MICROBIAL GLYCOSIDATION OF VALIDAMYCINS. II THE PREPARATION OF α AND β-D-GLUCOSIDE ANALOGS OF VALIDAMYCINS

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(Received for publication February 29, 1980)

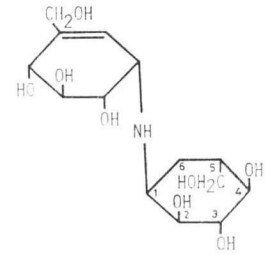
Validamycins, being curative fungicides, are particularly useful for the control of plant diseases caused by Basidiomycetes belonging to the *Corticiaceae*. They are especially effective against sheath blight of rice plants and some soil-borne diseases.

The proposed structure of validamycin A[1] has been revised[2] and structure-activity relationships in the validamycin group have been elucidated.[3] Validamycins are unique aminoglycoside antibiotics containing D-glucose in their structures. Validamycins A and D contain one molecule while validamycins C, E and F contain two molecules, respectively, of this sugar. The validamycins differ from one another in the site and/or type of glycosidic attachment to validoxylamine A (Fig. 1) which is a common moiety. Validamycin B has one molecule of β-D-glucose attached to validoxylamine B.[3]

Differences in the glycosidic component are considered to be of biological significance because the activities of these compounds differ by up to 1,000-fold[3]. For this reason it was of interest to prepare glycosidic analogs of the validamycins and investigate their biological properties.

We have reported on the formation of a few glycosidic analogs, β-D-galactosyl-validoxylamine A (Compound L-I), di-β-D-galactosyl-validoxylamine A (Compound L-II) and β-D-galactosyl-validamycin A (Compound L-III) by transglycosidation brought about by *Rhodotorula marina*[4]. We have now prepared new analogs, α and β-D-glucosides of validoxylamine A, with strains of *Rhodotorula* sp., different from *Rhodotorula marina*. The present paper deals with the isolation and characterization of α-D-glucosyl-validoxylamine A (Compounds M-I, II, IV, V) and β-D-glucosyl-validoxylamine A (Compounds C-I, II, III, IV).

Fig. 1. Structure of validoxylamine A(VA-A) and relationships within validamycin group.



Validamycin A (VM-A): 4-O-β-D-Glucopyranosyl-VA-A
Validamycin D: α-D-Glucopyranosyl-VA-A
Validamycin C, E, F: α-D-Glucopyranosyl-VM-A

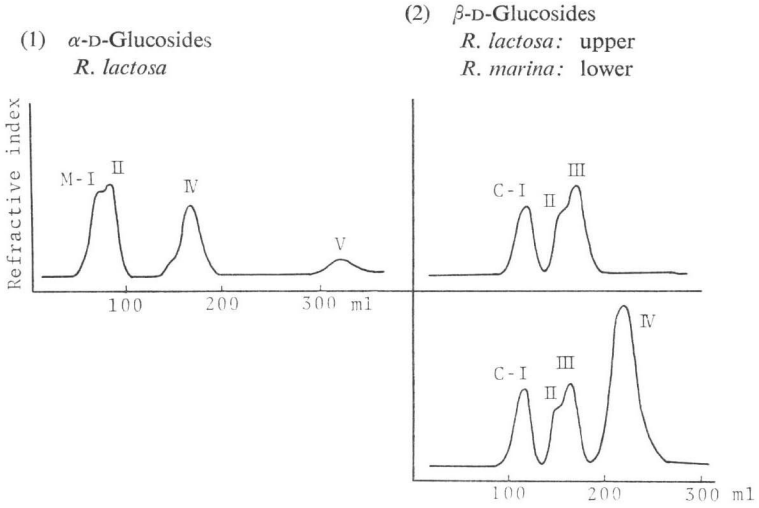
Rhodotorula lactosa IFO 1424 and *R. marina* IFO 1421 were cultured with shaking at 27°C for 48 hours in a medium (5 liters) composed of 1% glucose, 0.3% $(NH_4)_2SO_4$, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$ and 0.05% yeast extract (pH 5.7). The cells were collected by centrifugation at $10^4 \times g$ for 20 minutes, washed with 0.1 M phosphate buffer (pH 6.0) and suspended in the same buffer (500 ml). The suspensions were used as intact cell preparations in the following α and β-transglycosidations.

(1) α-Glucosidation of validoxylamine A

The reaction mixture (1 liter) containing validoxylamine A (1 g), maltose (10 g) and the cell suspension of *R. lactosa* IFO 1424 was incubated at 27°C under shaking conditions. The glycosidation process was followed by thin-layer chromatography (silica gel, *n*-PrOH - AcOH - H_2O, 4:1:1) and gas-liquid chromatography (trimethylsilyl derivatives, 5% silicone OV-17 on Chromosorb)[3]. At the time of maximum accumulation of the products (24 hours), the reaction mixture was filtered and passed through a column of Dowex 50W × 8 (H form, 100 ml), which was eluted with 0.5 N aqueous ammonia. After concentration to dryness, the residue was chromatographed on a column of Dowex 1 × 2 (OH form, 100 ml) and a column was developed with water to give five components: validoxylamine A and compounds M-I, II, IV and V in order of elution from the column. Compounds M-I and II overlapped slightly and the overlap fractions were chromatographed again on Dowex 50W × 8 (60 ml) equili-

* Takeda Chemical Industries, Ltd.

Fig. 2. Analytical chromatography of the α and β-D-glucosides (Dowex 1×2; OH form; 200~400 mesh; 70 cm×10 mm I. D.).



brated with 0.1 M pyridine-acetic acid buffer, pH 6.0, and eluted with the same buffer. In this way each component was completely resolved. Finally each component was rechromatographed on a Dowex 1×2 column (OH form, 50 ml) to obtain homogeneous samples of compounds M-I (80 mg), II (110 mg), IV (100 mg) and V (30 mg).

(2) β-Glucosidation of validoxylamine A

The reaction mixture (1 liter) containing validoxylamine A (1 g), cellobiose (10 g), and the cell suspension of *R. lactosa* IFO 1424 was incubated under the same conditions as described above and treated by the same procedures. Compounds C-I (60 mg), II (55 mg) and III (140 mg) were obtained.

The reaction using cells of *R. marina* IFO 1421 gave compound C-IV (240 mg) in addition to compounds C-I (70 mg), II (50 mg) and III (90mg).

Results and Discussion

Rhodotorula lactosa IFO 1424 and *Rhodotorula marina* IFO 1421 have the ability to convert validoxylamine A to new glucosidic analogs of validamycins by transglycosidation. The bioconversion products were analysed by the chromatography on Dowex 1×2 using water as eluant (Fig. 2) and by gas-liquid chromatography.

Both strains formed compounds C-I, II and III in the presence of cellobiose as a β-glucosyl donor. However, only *R. marina* was able to form compound C-IV. *R. lactosa*, but not *R. marina*, was able to form compounds M-I, II, IV and V in the presence of maltose as an α-glucosyl donor. It seems that there are differences in specificity between the β-glucosidases of the two strains and that *R. marina* rarely produces the α-glucosidase concerned with the transglycosidation.

All compounds were isolated as amorphous colorless materials which are distinguishable from each other and homogeneous by chromatographic procedures.

Each of them gave one molecule of validoxylamine A and D-glucose by acid hydrolysis (2 N H_2SO_4, 80°C, 8 hours). Other characterization data are summarized in Table 1.

The ^{1}H-NMR signals for the anomeric protons showed that the compound C series were β-D-glucosides and the compound M series were α-D-glucosides. The optical rotations supported these assignments. Compounds M-II and IV, as well as compounds C-III and C-IV, were not resolved by gas-liquid chromatography, but as shown in Fig. 2 these compounds are distinguishable from each other by liquid chromatography. From the data described above, compounds M-II and C-III were identified as validamycins D and A, respectively. The ^{1}H-NMR spectra confirmed these structures.

The new compounds showed low activity against *Rhizoctonia solani* by the "dendroid-test method"[5] (Table 2). β-Glucoside analogs were more active than α-glucoside analogs and the ef-

Table 1. Properties of the glucosidic analogs of validamycins.

	Synonym	$[\alpha]_D^{25}$ (H_2O)	tlc*	NMR (D_2O)** anomeric proton
Compound C-I	β-D-glc-VA-A	+88.1	0.24	δ 4.54 (J=8.0 Hz)
C-II	〃	+132.6	0.24	δ 4.52 (J=8.0 Hz)
C-III	〃	+108.3	0.24	δ 4.52 (J=8.0 Hz)
C-IV	〃	+105.6	0.24	δ 4.64 (J=8.0 Hz)
Compound M-I	α-D-glc-VA-A	+182.6	0.24	δ 4.92 (J=3.5 Hz)
M-II	〃	+170.8	0.24	δ 4.92 (J=3.5 Hz)
M-IV	〃	+160.0	0.24	δ 5.00 (J=3.5 Hz)
M-V	〃	+178.0	0.28	δ 4.96 (J=3.5 Hz)

* Rf value, Solvent: *n*-propanol - acetic acid - water (4: 1: 1); silica gel G
** at 100 MHz with sodium 2,2-dimethyl-2-silapentane-5-sulfonate standard

Table 2. Minimum concentration causing abnormal branching at the tips of hyphae of *Rhizoctonia solani*.

Analogs	Minimum concentration (mcg/ml)
Compound C-I	1.25
C-II	1.56
C-III	0.0125
C-IV	0.625
Compound M-I	50
M-II	50
M-IV	2.5
M-V	5.0

Table 3. Inhibitory effect of validamycin analogs on the spread of sheath blight on rice plants.

Analogs (10 ppm)	Expansion rate (%)*
Compound C-I	46
C-II	61
C-III	18
C-IV	21
Compound M-I	62
M-II	57
M-IV	72
M-V	not tested
Validamycin A	19
Untreated	100

* Rice plants were planted in a 1/5000-are Wagner pot and inoculated. After 4 days, pots were treated with drugs; the expansion rate was calculated 10 days after application of the drugs.

$$\text{Expansion rate} = \frac{\text{average length of lesion per stem treated with drugs}}{\text{average length of lesion per untreated stem}} \times 100\ (\%)$$

fect of the different sites of glucosidic linkage on the activity was considerably less with the former type. However, by the green house test the compounds, particularly compound C-IV, showed considerably more activity in the control of sheath blight of rice plants than predicted from the *in vitro* test (Table 3). This discrepancy is attributed to differences in the stability of each compound to microbial attack when on the rice plants.

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