MICROBIAL GLYCOSIDATION OF VALIDAMYCINS

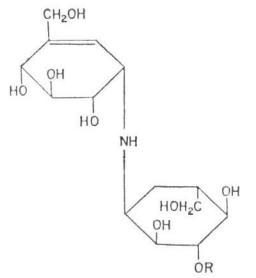
Sir:

The chemical structure of validamycin A[1] and the structure-activity relationships[2] of the validamycin group were reported in previous papers. Validamycins A, C, D, E and F contain validoxylamine A as a common moiety, but differ from one another in the number, the site and/or the type of glucosidic attachment to validoxylamine A.

We felt it worthwhile to produce glycosidic analogs of the validamycins in search of derivatives possessing resistance to microbial attack[3] and more potent activity. We have investigated the microbial transglycosidation of validamycins by *Rhodotorula marina* IFO-1421, and obtained two products in addition to the compound previously reported.[3]

We now report on the isolation and characterization of these new validamycin analogs.

Fig. 1. Structures of validoxylamine A and validamycin A.



Validoxylamine A: R=H
Validamycin A: R= β-D-Glucose

Rhodotorula marina IFO-1421 was cultured with shaking at 27°C in the medium (2 liters) containing validoxylamine A (2 g) as an acceptor and lactose (20 g) as a β-galactosyl donor. The composition of the medium was as follows: validoxylamine A 0.1%, lactose 1%, $(NH_4)_2SO_4$ 0.3%, KH_2PO_4 0.1% and yeast extract 0.05% (pH 5.7).

The glycosidation process was followed by thin-layer chromatography (silica gel, *n*-PrOH - AcOH - H_2O, 4 : 1 : 1) and gas liquid chromatography (TMS derivatives, 5% silicone OV-17 on chromosorb).[2]

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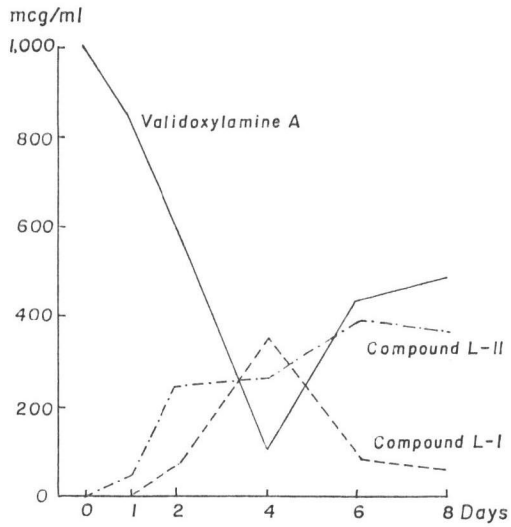
Fig. 2. Production of compounds L-I and -II by *Rhodotorula marina*.



Production of the analogs in the cultured broth is shown in Fig. 2. Starting on the first day of the culture, validoxylamine A decreased gradually, while the production of compounds L-I and L-II increased. The maximum accumulations of compounds L-I and L-II were observed on day 4 and on day 6, respectively.

The 4-day culture was filtered at pH 8 and passed through a column of activated carbon (200 ml), which was eluted with water containing 7% *n*-butanol. The eluate was adsorbed on a column of Dowex 50W×2 (H form, 200 ml) and the column 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) using H_2O as a developing solvent. The differential refractometer was used for monitoring the elution and each fraction was checked by gas liquid chromatography. Repeated separation yielded pure compounds L-I (820 mg) and L-II (840 mg).

Compound L-I: Colorless amorphous powder. $[\alpha]_D^{21}+115.6°$ (*c* 1, H_2O), Anal. Calcd. for $C_{20}H_{35}NO_{13}\cdot H_2O$: C, 46.60; H, 7.24; N, 2.72. Found: C, 46.65; H, 7.14; N, 2.91, NMR (D_2O) δ4.56 (d, J=8 Hz, anomeric proton), TLC Rf 0.25.

Acid hydrolysis (2 N H_2SO_4, 80°C, 8 hours) of compound L-I afforded an equivalent mole of

validoxylamine A and D-galactose. Compound L-I was identified as β-D-galactosyl-validoxylamine A, which was reported previously.[3]

Compound L-II: Colorless amorphous powder. $[\alpha]_D^{21}+96.8°$ (*c* 1, H_2O), Anal. Calcd. for $C_{26}H_{45}NO_{18}\cdot H_2O$: C, 46.08; H, 6.99; N, 2.07. Found: C, 46.42; H, 7.29; N, 2.22, NMR (D_2O) δ 4.48, 4.60 (d, J=8 Hz, anomeric protons), TLC Rf 0.14. Compound L-II gave one mole of validoxylamine A and two moles of D-galactose by acid hydrolysis. Compound L-II appears to be di-β-D-galactosyl-validoxylamine A.

In the same procedure as described above, the addition to the cultured broth of validamycin A (2 g) as an acceptor, yielded compound L-III (620 mg).

Compound L-III: Colorless amorphous powder. $[\alpha]_D^{21}+88.0°$ (*c* 1, H_2O), Anal. Calcd. for $C_{26}H_{45}NO_{18}\cdot H_2O$: C, 46.08; H, 6.99; N, 2.07. Found: C, 46.20; H, 7.15; N, 2.13, NMR (D_2O) δ 4.54, 4.61 (d, J=8 Hz, anomeric protons), TLC Rf 0.20. Compound L-III gave one mole of validoxylamine A and one mole each of D-glucose and D-galactose by acid hydrolysis (2 N H_2SO_4, 80°C, 8 hours) and gave validamycin A as a partial hydrolysis product by mild acid hydrolysis (0.5 N H_2SO_4, 80°C, 3 hours). A consideration of the data indicates compound L-III to be β-D-galactosyl-validamycin A.

Compounds L-I, -II and -III, as well as validamycins did not inhibit the growth of bacteria and fungi.

As shown in Table 1* these compounds showed poor activity against *Pellicularia sasakii* by the "dendroid-test method".[4] However they have shown considerable activity by the green house test (1~1/10 activity of validamycin A). The results of the green house test will be reported by O. WAKAE and his co-workers elsewhere. The pronounced differences of activity in the *in vitro* and *in vivo* test seem to be a worthwhile subject to investigate.

As to the mechanism of β-transglycosidation, P. A. J. GORIN and co-workers have studied extensively the β-galactosidase of *Sporobolomyces singularis*.[5] They reported on the "group transfer system" by a hydrolase which is capable of catalyzing transfer as well as hydrolysis. After induction of the β-galactosidase by lactose, simultaneous protonation of the glycosidic oxygen and nucleophilic attack take place in the active site of the enzyme, respectively, and the intermediate complex formed can react with a new alcohol to give a new β-galactoside.

Table 1. The minimum concentration causing abnormal branching at the tops of the hyphae of *Pellicularia sasakii*

Validamycin analogs	Minimum concentration (mcg/ml)
Compound L-I	25
L-II	200
L-III	25
Validamycin A	0.006

We have further observed that resting cells of *Rhodotorula marina* catalyze the β-D-galactosyl transfer of lactose to validoxylamine A and validamycin A, resulting in the formation of compounds L-I, -II and compounds L-I, -II, -III, respectively. The yeast does not transfer free D-galactose to validoxylamine A in the same system. It seems likely that compound L-I, -II and -III are formed by the same mechanism as described above.

These results indicate a valuable approach to the formation of glycosides with desirable activity. Studies are continuing on the production of novel antibiotics using this technique.

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