Communications to the Editor

VALIDAMYCIN G AND VALIDOXYLAMINE G, NEW MEMBERS OF THE VALIDAMYCINS

Sir:

The validamycins produced by *Streptomyces hygroscopicus* subsp. *limoneus* comprise the eight components, validamycins A~F and validoxylamines A and B[1,2]. Validoxylamine A, the common aglycone of validamycins A, C, D, E and F, consists of two kinds of amino-cyclitols, valienamine and validamine. Validoxylamine B, the aglycone of validamycin B, consists of valienamine and hydroxyvalidamine.

We previously isolated a new aminocyclitol, valiolamine from the fermentation broth of *Streptomyces hygroscopicus* subsp. *limoneus*, which is a producer of the validamycins, together with valienamine, validamine and hydroxyvalidamine, and reported that valiolamine has more potent α-glucosidase inhibitory activity against porcine intestinal sucrase, maltase and isomaltase than the others[3]. This prompted us to find new validamycins containing valiolamine as a constituent of the molecule. In this paper, we report the isolation, structure determination and biological properties of the new components, validoxylamine G and its β-D-glucoside, validamycin G.

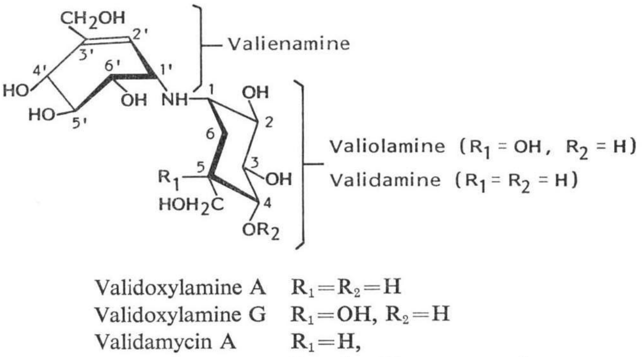
The crude validamycins (1.2 kg), prepared from the fermentation broth as previously reported[1,4], were chromatographed on a column of Dowex 1-X2 (OH⁻ form) and eluted with H_2O to give ten components, validoxylamines A, G and B, and validamycins D, A, G, C, B, F and E in order of elution from the cloumn. The validoxylamine G and validamycin G fractions were further chromatographed on a Dowex 50W-X8 column (elution with 0.2 M pyridine - acetate buffer, pH 6.0). Finally, each component was rechromatographed on Dowex 1-X2 column (OH⁻ form) to obtain homogeneous validoxylamine G (2.2 g) and validamycin G (0.1 g). The Rf values of these new components on silica gel TLC in comparison with the validamycins are given in Table 1.

Validoxylamine G: Colorless amorphous; $[\alpha]_D^{25}$ +118.6° (*c* 1.0, H_2O); *Anal* Calcd for $C_{14}H_{25}NO_9 \cdot H_2O$: C 45.52, H 7.36, N 3.79, Found: C 45.83, H 7.45, N 3.62.

Validamycin G: Colorless amorphous; $[\alpha]_D^{25}$ +52.8° (*c* 0.5, H_2O); *Anal* Calcd for $C_{20}H_{35}NO_{14} \cdot H_2O$: C 45.19, H 7.01, N 2.63, Found: C 45.31, H 7.19, N 2.47. The ^{13}C NMR spectral data of validoxylamine G and validamycin G are listed in Table 2. In this report, the position number of the carbon atoms of the validamycins are shown in Fig. 1.

The molecular formulae were established by elementary analysis, and ^{13}C and ^1H NMR spectrometry as $C_{14}H_{25}NO_9$ for validoxylamine G and $C_{20}H_{35}NO_{14}$ for validamycin G. The ^{13}C NMR spectrum of validoxylamine G revealed

Fig. 1.



Validoxylamine A $R_1=R_2=H$
Validoxylamine G $R_1=OH$, $R_2=H$
Validamycin A $R_1=H$,
$R_2=\beta$-D-Glucopyranosyl
Validamycin G $R_1=OH$,
$R_2=\beta$-D-Glucopyranosyl

Scheme 1.

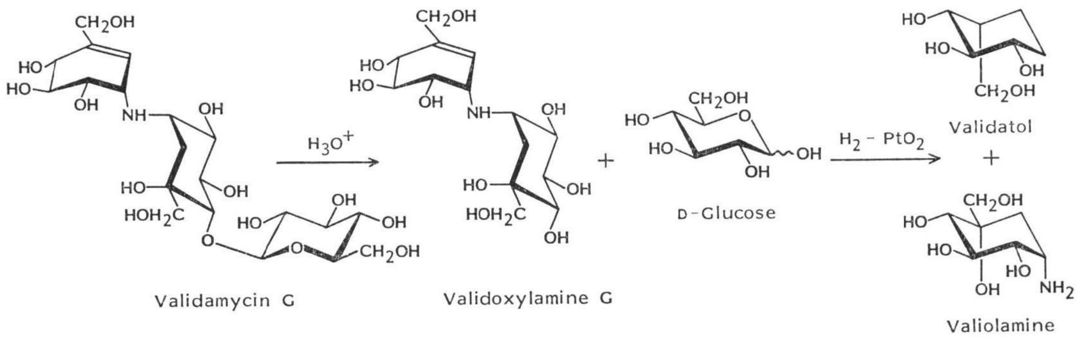


Table 1. Rf values of validamycins.

Compounds	I	II
Validoxylamine A	0.30	0.40
Validoxylamine B	0.37	0.32
Validoxylamine G	0.23	0.24
Validamycin A	0.20	0.29
Validamycin B	0.29	0.23
Validamycin C	0.14	0.14
Validamycin D	0.20	0.21
Validamycin E	0.14	0.21
Validamycin F	0.14	0.21
Validamycin G	0.18	0.14

Solvent system: I; PrOH - AcOH - H_2O (4: 1: 1).
II; BuOH - MeOH - $CHCl_3$ - concd NH_4OH (4: 5: 2: 5).
Silica gel TLC: Kieselgel 60 F_{254} (Merck).

14 signals ($CH_2 \times 3$, $CH \times 9$ and $C \times 2$) as shown in Table 2. Acid hydrolysis of validamycin G using Dowex 50W-X8 (H^+ form) gave equimolar amounts of D-glucose and validoxylamine G. Validoxylamine G can be degraded further into validatol[5,6], the cyclitol unit, and valiolamine by hydrogenolysis with PtO_2 - H_2. A similar degradation scheme has been used for validamycin A (Scheme 1). These experiments show that validoxylamine G consists of valiolamine and valienamine (or 1-*epi*-valienamine), and validamycin G is a mono-D-glucoside of validoxylamine G.

Acetylation of validoxylamine G and validamycin G with acetic anhydride in pyridine afforded an octaacetate ($C_{30}H_{41}NO_{17}$, EI-MS *m/z* 687 (M)) and an undecaacetate ($C_{42}H_{57}NO_{25}$, FAB-MS *m/z* 976 (M+1)), respectively. The ^1H NMR spectral data (400 MHz, in $CDCl_3$) are summarized in Table 3. All the protons could be assigned by detailed decoupling experiments.

Table 2. ^{13}C NMR spectral data of validoxylamine G and validamycin G.

Carbon	Chemical shifts δ (ppm)*	
	Validoxylamine G	Validamycin G
C-1	54.4 d	54.5 d
C-2	75.5 d	75.4 d
C-3	74.3 d	72.9 d
C-4	76.6 d	86.0 d
C-5	78.4 s	79.0 s
C-6	31.4 t	31.8 t
C-7	67.8 t	67.1 t
C-1′	56.7 d	56.4 d
C-2′	125.3 d	125.0 d
C-3′	142.2 s	142.1 s
C-4′	75.6 d	75.8 d
C-5′	74.3 d	74.3 d
C-6′	72.4 d	72.5 d
C-7′	64.3 t	64.3 t
C-1″		105.8 d
C-2″		76.2 d
C-3″		78.5 d
C-4″		72.1 d
C-5″		78.7 d
C-6″		63.2 t

* δ (ppm) from internal sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS).

In the data for validoxylamine G octaacetate, the singnal for the vinyl proton (H-2′) at δ 5.99 appeared a doublet with $J_{2',1'}=5.1$ Hz. The magnitude of coupling constant suggests that H-1′ of the valienamine moiety has the same pseudo-equatorial configuration as that of validoxylamine A[7]. Thus, the structure of validoxylamine G was elucidated as [(1*S*)-(1,4,6/5)-4,5,6-trihydroxy-3-hydroxymethyl-2-cyclohexenyl]-[(1*S*)-(1,2,4,5(*OH*)/3,5)-2,3,4,5-tetrahydroxy-5-(hydroxymethyl)cyclohexyl]amine (Fig. 1).

The ^1H NMR spectrum of validamycin G

Table 3. ^{1}H NMR data of validoxylamine G octaacetate and validamycin G undecaacetate in $CDCl_3$ (400 MHz).

Validoxylamine G octaacetate			Validamycin G undecaacetate		
Proton	Chemical shift*	Multiplicity and coupling constant (Hz)	Proton	Chemical shift*	Multiplicity and coupling constant (Hz)
H-1	3.547	dt J=2.9, 3.1, 4.6	H-1	3.426	dt J=2.7, 3.1, 4.4
H-2	5.018	dd J=4.6, 10.3	H-2	4.928	dd J=4.4, 9.9
H-3	5.613	t J=10.3	H-3	5.522	t J=9.9
H-4	5.069	d J=10.3	H-4	3.665	d J=9.9
H-6	1.688	dd J=2.9, 15.4	H-6	1.492	dd J=2.7, 15.3
	1.942	dd J=3.1, 15.4		1.895	dd J=3.1, 15.3
H-7	3.653	ABq J=11.4	H-7	3.922	ABq J=11.1
	4.042			4.127	
H-1′	3.603	m	H-1′	3.598	m
H-2′	5.995	d J=5.1	H-2′	5.973	d J=4.6
H-4′	5.401	d J=6.8	H-4′	5.383	d J=6.6
H-5′	5.435	dd J=6.8, 10.6	H-5′	5.417	dd J=6.6, 10.2
H-6′	4.983	dd J=5.1, 10.6	H-6′	4.985	dd J=5.1, 10.2
H-7′	4.384	ABq J=13.4	H-7′	4.371	ABq J=13.3
	4.601			4.599	
NH	6.594	s	H-1″	4.498	d J=9.1
$COCH_3$	2.017	s	H-2″	4.967	t J=9.1
	2.048	s	H-3″	5.163	t J=9.1
	2.067	s	H-4″	5.089	t J=9.1
	2.068	s	H-5″	3.632	ddd J=2.3, 4.1, 9.1
	2.071×2	s	H-6″	4.037	dd J=2.3, 12.5
	2.078	s		4.404	dd J=4.1, 12.5
	2.139	s	NH	5.870	br s
			$COCH_3$	1.989	s 2.061 s
				2.007	s 2.065 s
				2.032	s 2.083 s
				2.044	s 2.102 s
				2.052	s 2.141 s
				2.058	s

* Chemical shifts in δ values (ppm down-field from internal TMS).

Table 4. Minimum concentration causing abnormal branching at the tips of hyphae of *Rhizoctonia solani*.

Compound	Minimum concentration (μg/ml)
Validamycin A	0.01
Validamycin G	0.50
Validoxylamine A	1.00
Validoxylamine G	2.50

undecaacetate revealed the presence of a β-anomeric proton as a doublet at δ 4.49 (1H, J=9.1 Hz). A comparison of the NMR spectral data of validoxylamine G octaacetate and validamycin G undecaacetate suggests that the hydroxyl group on C-4 of the valiolamine moiety is substituted by a β-D-glucosyl group, since an appreciable up-field shift of 1.40 ppm was observed in the position of H-4 in the spectrum of

Table 5. Molar concentration required to give 50% inhibition against porcine intestinal α-glucosidases.

Compound	Maltase	Sucrase	Isomaltase
Validamycin A	$>1.0\times10^{-3}$	$>1.0\times10^{-3}$	$>1.0\times10^{-3}$
Validamycin G	$>1.0\times10^{-3}$	1.1×10^{-4}	$>1.0\times10^{-3}$
Validoxylamine A	$>1.0\times10^{-3}$	$>1.0\times10^{-3}$	$>1.0\times10^{-3}$
Validoxylamine G	1.0×10^{-3}	8.8×10^{-6}	1.7×10^{-4}
Valiolamine	2.2×10^{-6}	4.9×10^{-8}	2.7×10^{-6}

Substrate: 50 mM maltose, 50 mM sucrose, 40 mM isomaltose.

validamycin G undecaacetate. Therefore the structure of validamycin G was shown to be 4-*O*-β-D-glucopyranosylvalidoxylamine G (Fig. 1).

Validamycin G and validoxylamine G are less active against *Rhizoctonia solani* by the "dendroid-test method"[8], as shown in Table 4. Validamycins A ~ F have poor activity against porcine intestinal α-glucosidases with IC_{50} values greater than 10^{-3} M. The activity of the new components are compared in Table 5.

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