VALIDAMYCIN H, A NEW PSEUDO-TETRASACCHARIDE ANTIBIOTIC

Sir:

Validamycin A has been used to control some diseases caused by *Rhizoctonia solani*, particularly sheath blight of rice plants. Validamycin A is the major component of the validamycin complex produced by *Streptomyces hygroscopicus* subsp. *limoneus*. The validamycin complex comprises the ten components, validamycins A ~ G and validoxylamines A, B and G (Fig. 1)[1~3]. In the course of purification of pseudo-tetrasaccharides, validamycins C, E and F, we found a new pseudo-tetrasaccharide, validamycin H.

In this communication, we report the isolation, structure elucidation and biological activity against *R. solani* of the new pseudo-tetrasaccharide, validamycin H.

The crude validamycin complex (120 g), prepared from the fermentation broth as previously reported[1,4], was passed through a column of Amberlite IR-120B (H^+) and the column was eluted with 0.5 N ammonium hydroxide. The eluate was concentrated and applied onto a column of Amberlite CG-50 (NH_4^+). The effluent and washings were concentrated and lyophilized to give the validamycin complex (59 g). Approximately 10 g portions of the validamycin complex were chromatographed on a column (4.2 × 90 cm) of Bio-Gel P-2 (200 ~ 400 mesh) and tetrasaccharides fraction was pooled. This fraction was further purified on a column of Dowex 1-X2 (OH^-, 100 ml) and eluted with H_2O to give validamycins C (61 mg), H (60 mg)

Table 1. ^{13}C NMR chemical shifts[a] of validamycin H.

Carbon	Validamycin A	Validamycin H
C-1	56.53 d	56.60 d
C-2	75.96 d	75.96 d
C-3	75.54 d	75.80 d
C-4	87.10 d	87.75 d
C-5	40.23 d	40.20 d
C-6	29.67 t	29.64 t
C-7	64.60 t	64.71 t
C-1′	55.20 d	55.22 t
C-2′	125.97 d	126.05 d
C-3′	141.98 s	142.05 s
C-4′	74.28 d	74.34 d
C-5′	76.49 d	76.54 d
C-6′	72.23 d	72.29 d
C-7′	64.37 t	64.41 t
C-1″	105.67 d	105.87 d
C-2″	76.22 d	76.17 d
C-3″	78.43 d	78.37 d
C-4″	72.23 d	72.33 d
C-5″	78.80 d	77.58 d
C-6″	63.34 t	71.61 t
C-1‴		105.51 d
C-2‴		75.96 d
C-3‴		78.45 d
C-4‴		72.44 d
C-5‴		78.84 d
C-6‴		63.58 t

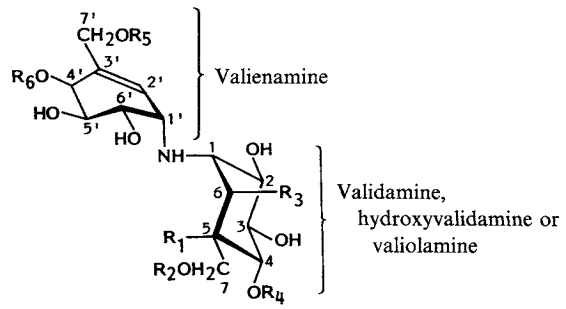
[a] δ (ppm) from 3-(trimethylsilyl)propionate (TSP) in D_2O.

Table 2. ^1H NMR data of validamycins A and H (δ in ppm[a], J in Hz).

Proton	Validamycin A	Validamycin H
1-H	3.287 (br q)	3.290 (br q)
2-H	3.633 (dd, J=9.5, 4.0)	3.639 (dd, J=10.1, 4.0)
3-H	3.748 (t, J=9.5)	3.757 (dd, J=10.1, 9.3)
4-H	3.515 (dd, J=10.0, 9.5)	3.515 (dd, J=10.5, 9.3)
5-H	2.097 (m)	2.103 (m)
6-H_{ax}	1.366 (ddd, J=14.5, 13.0, 2.8)	1.364 (ddd, J=14.8, 13.2, 2.8)
6-H_{eq}	1.960 (dt, J=14.5, 3.2)	1.967 (dt, J=14.8, 3.2)
7-$H_{a,b}$	3.788 (d, J=4.3)	3.786 (d, J=4.6)
1′-H	3.382 (br t, J=4.9)	3.367 (br s)
2′-H	6.046 (dq, J=4.9, 1.5)	6.048 (dq, J=5.0, 1.5)
4′-H	4.095 (br d, J=5.7)	4.097 (br d, J=5.9)
5′-H	3.633 (dd, J=9.0, 5.7)	3.636 (dd, J=9.5, 5.9)
6′-H	3.635 (dd, J=9.0, 4.0)	3.636 (dd, J=9.5, 3.9)
7′-H_a	4.138 (br d, J=13.9)	4.139 (br d, J=13.8)
7′-H_b	4.251 (dq, J=13.9, 1.0)	4.253 (dq, J=13.8, 1.0)
1″-H	4.528 (d, J=8.0)	4.532 (d, J=8.0)
2″-H	3.347 (dd, J=9.1, 8.0)	3.367 (dd, J=9.3, 8.0)
3″-H	3.524 (t, J=9.1)	3.513 (t, J=9.3)
4″-H	3.435 (dd, J=9.5, 9.1)	3.497 (dd, J=9.8, 9.3)
5″-H	3.497 (m)	3.675 (m)
6″-H_a	3.744 (dd, J=12.3, 5.8)	3.874 (dd, J=12.4, 6.3)
6″-H_b	3.916 (dd, J=12.3, 2.1)	4.224 (dd, J=12.4, 2.1)
1‴-H		4.512 (d, J=8.0)
2‴-H		3.332 (dd, J=9.3, 8.0)
3‴-H		3.513 (t, J=9.3)
4‴-H		3.402 (dd, J=9.8, 9.3)
5‴-H		3.463 (m)
6‴-H_a		3.675 (dd, J=12.4, 5.6)
6‴-H_b		3.922 (dd, J=12.4, 2.1)

[a] See footnote in Table 1.

Fig. 1. Structure of the validamycin complex.



Validoxylamine A	R_1=H	R_2=H	R_3=H	R_4=H	R_5=H	R_6=H
Validoxylamine B	R_1=H	R_2=H	R_3=OH	R_4=H	R_5=H	R_6=H
Validoxylamine G	R_1=OH	R_2=H	R_3=H	R_4=H	R_5=H	R_6=H
Validamycin A	R_1=H	R_2=H	R_3=H	R_4=β-D-Glc	R_5=H	R_6=H
Validamycin B	R_1=H	R_2=H	R_3=OH	R_4=β-D-Glc	R_5=H	R_6=H
Validamycin C	R_1=H	R_2=H	R_3=H	R_4=β-D-Glc	R_5=α-D-Glc	R_6=H
Validamycin D	R_1=H	R_2=α-D-Glc	R_3=H	R_4=H	R_5=H	R_6=H
Validamycin E	R_1=H	R_2=H	R_3=H	R_4=α-D-Glc(1-4)-β-D-Glc	R_5=H	R_6=H
Validamycin F	R_1=H	R_2=H	R_3=H	R_4=β-D-Glc	R_5=H	R_6=α-D-Glc
Validamycin G	R_1=OH	R_2=H	R_3=H	R_4=β-D-Glc	R_5=H	R_6=H
Validamycin H	R_1=H	R_2=H	R_3=H	R_4=β-D-Glc(1-6)-β-D-Glc	R_5=H	R_6=H

Glc: Glucopyranosyl.

and a mixture (238 mg) of validamycins E and F in order of elution from the column. The Rf values of validamycins C, E, F and H on TLC (Silica gel $60F_{254}$, Merck) were all 0.14 with PrOH-AcOH-H_2O (4:1:1) as the developing system, and 0.14, 0.21, 0.21, 0.21 with BuOH-MeOH-$CHCl_3$-concd NH_4OH (4:5:2:5).

Validamycin H: Colorless amorphous; $[\alpha]_D^{25}$ +74.9° (*c* 1, H_2O). Acid hydrolysis of validamycin H using Dowex 50W-X8 (H^+) gave D-glucose and validoxylamine A as an aglycone.

The structure of validamycin H was determined by ^{13}C NMR, ^1H NMR, DEPT, ^{13}C-^1H COSY, ^1H-^1H COSY and NOESY experiments with a Jeol JNM-GX400 spectrometer in comparison with the data of validamycin A. The ^{13}C and ^1H NMR spectral data of validamycins A and H are listed in Tables 1 and 2, respectively. The coupling constants of the anomeric protons 1″-H (J=8.0 Hz) and 1‴-H (J=8 Hz) in ^1H NMR spectrum of validamycin H demonstrated the modes of glucosidic linkages as both β. As shown in Tables 1 and 2, validamycins A and H exhibited the identical chemical shifts to each other for the chemical shifts of validoxylamine A moiety. In addition, the further glucoside formation for a pseudo-trisaccharide (β-D-glucopyranosylvalidoxylamine A) produced a 8.27-ppm downfield shift for C-6″ in ^{13}C NMR, 0.130 and 0.308 ppm downfield shifts for 6″-H in ^1H NMR. By NMR studies mentioned above and the NOE between 1″-H and 4-H, and 1‴-H and 6″-H_b observed by NOESY, the positions of glucosidic linkages were determined at C-4 and C-6″, respectively. Therefore the structure of validamycin H was shown to be 6″-*O*-β-D-glucopyranosylvalidamycin A (Fig. 1).

Table 3. Effect of the validamycin complex on *Rhizoctonia solani* in "dendroid-test method".

Compound	Dendroid-test method (μg/ml)[a]
Validoxylamine A	1.00
Validoxylamine B	50
Validoxylamine G	2.50
Validamycin A	0.01
Validamycin B	0.50
Validamycin C	10
Validamycin D	25
Validamycin E	0.01
Validamycin F	0.01
Validamycin G	0.50
Validamycin H	0.05

[a] Minimum concentration causing abnormal branching.

Validamycin H exhibited 5-fold weaker activity against *R. solani* by the "dendroid-test method"[5] than validamycins E and F, which are α-D-glucopyranosylvalidamycin A (Table 3). The glucoside introduction into the hydroxymethyl group of validoxylamine A (validamycins C and D) causes the reduction of activity against *R. solani*. The reduction of activity in validamycin H may be due to the suppression of permeability of the antibiotic into the pathogen[6] by the glucoside introduction into the hydroxymethyl group (C-6″) of the glucosyl residue.

Acknowledgments

We express our thanks to Dr. HARUHISA KIZU (School of Pharmacy, Hokuriku University) for measurement of NMR spectra.

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(Received March 3, 1990)

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