ALL EIGHT POSSIBLE MONO-β-D-GLUCOSIDES OF VALIDOXYLAMINE A

I. PREPARATION AND STRUCTURE DETERMINATION

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Validamycin A is the major and most active compound among the validamycin complex. Since the site of β-glucosidic attachment to validoxylamine A (**1**) was expected to affect the activity against the pathogenic fungus, *Rhizoctonia solani*, all eight possible mono-β-D-glucosides of **1** were prepared. 2-*O*-, 4-*O*-, 4′-*O*-, and 7′-*O*-β-D-glucopyranosylvalidoxylamine A (**2**, **4**, **6** and **9**, respectively) were prepared by microbial β-glycosylation of **1** with strains of *Rhodotorula* sp. 7-*O*- and 6′-*O*-β-D-glucopyranosylvalidoxylamine A (**5a** and **8a**, respectively) were prepared semi-synthetically through microbial formation of 7-*O*-β-D-glucopyranosylvalidamine (**10**), oxidation of the primary amine of **10** to a ketone, and coupling of the ketone derivative with valienamine, and through microbial formation of 6-*O*-β-D-glucopyranosylvalienamine (**11**), and coupling of **11** with (2*R*)-(2,4/3,5)-2,3,4-trihydroxy-5-hydroxymethylcyclohexanone (**12**), respectively. 3-*O*- and 5′-*O*-β-D-glucopyranosylvalidoxylamine A (**3a** and **7a**, respectively) were chemically synthesized.

Validamycin A is the major and most active component of the validamycin complex produced by *Streptomyces hygroscopicus* subsp. *limoneus*[1] and has been used to control some diseases caused by *Rhizoctonia solani*, particularly sheath blight of rice plants. Validamycin A is a pseudo-trisaccharide antibiotic characterized by the novel structure of its aglycone, validoxylamine A (**1**), which consists of valienamine and validamine (Fig. 1).

We have previously reported the isolation of four mono-β-D-glucosides by microbial glycosylation of **1** with strains of *Rhodotorula* sp. and their activity[2]. Differences in the site of β-glucosidic attachment to **1** are considered to be biologically significance because the activity of these compounds differ by up to 100-fold in the "dendroid-test method"[3]. For this reason it is of much interest to prepare all eight possible mono-β-D-glucosides of **1** and investigate their biological activities.

In the present paper we describe the structure determination of four mono-β-D-glucosides obtained by microbial glycosylation of **1**, and the semi-synthesis and chemical synthesis of the other four mono-β-D-glucosides.

Results and Discussion

Structure Determination of Four Microbial Glycosylation Products

We have reported on the formation of four β-D-glucopyranosylvalidoxylamines A, compounds C-I, C-II, C-III and C-IV, by microbial glycosylation with strains of *Rhodotorula* sp.[2]. However, the sites of their β-glucosidic linkages have not been determined. We have determined the sites of the β-glucosidic linkages by ^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H-^{1}H COSY and ^{13}C-^{1}H COSY experiments with a Jeol JNM-GX 400 spectrometer. Their ^{1}H NMR and ^{13}C NMR assignments are listed in Tables 1 and 2.

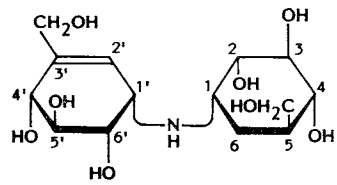
In the ^{13}C NMR spectrum of compound C-I, glucoside formation produced a 6.6-ppm downfield shift for the hydroxymethyl carbon at the allylic position in the valienamine unit (C-7′), a 2.8-ppm upfield

shift at C-3′ and a 3.2-ppm downfield shift at C-2′, which are consistent with the β and γ shifts of the olefinic carbons caused by glycosylation at the allylic position[4]. Therefore the site of glucosidic linkage was determined as C-7′, and the structure of compound C-I was shown to be 7′-*O*-β-D-glucopyranosylvalidoxylamine A (**9**).

The ^{13}C NMR spectra of compounds C-II and C-III displayed a 10.2-ppm downfield shift for C-2 and a 10.9-ppm downfield shift for C-4, respectively. Therefore the sites of the glycosylation in compounds C-II and C-III were determined at C-2 and C-4, respectively, and the structures of compounds C-II and C-III were shown to be 2-*O*-β-D-glucopyranosylvalidoxylamine A (**2**) and 4-*O*-β-D-glucopyranosylvalidoxylamine A (**4**, validamycin A), respectively.

In the ^{13}C NMR spectrum of compound C-IV, glucoside formation produced a 10.5-ppm downfield shift for the allylic methine carbon (C-4′), and a 2.0-ppm upfield shift and a 2.2-ppm downfield shift for the olefinic carbons (C-3′ and C-2′, respectively). These shifts are in good agreement with the results previously reported for trestatin

Fig. 1. Eight theoretically possible mono-β-D-glucosides of validoxylamine A.



Validoxylamine A (**1**)

2-*O*-β-D-Glucopyranosylvalidoxylamine A (**2**)
3-*O*-β-D-Glucopyranosylvalidoxylamine A (**3a**)
4-*O*-β-D-Glucopyranosylvalidoxylamine A (**4**: Validamycin A)
7-*O*-β-D-Glucopyranosylvalidoxylamine A (**5a**)
4′-*O*-β-D-Glucopyranosylvalidoxylamine A (**6**)
5′-*O*-β-D-Glucopyranosylvalidoxylamine A (**7a**)
6′-*O*-β-D-Glucopyranosylvalidoxylamine A (**8a**)
7′-*O*-β-D-Glucopyranosylvalidoxylamine A (**9**)

Table 1. ^1H NMR data of all eight possible mono-β-D-glucosides of validoxylamine A (δ in ppm, J in Hz).

Proton	**2** (Compound C-II)	**3a**	**4** (Compound C-III)	**5a**
1-H	3.592 (m)	3.374 (br s)	3.287 (br q)	3.289 (br s)
2-H	3.700 (dd, J=9.5, 4.4)	3.797 (dd, J=9.5, 4.0)	3.633 (dd, J=9.5, 4.0)	3.594 (dd, J=10.3, 4.0)
3-H	3.847 (t, J=9.5)	3.844 (t, J=9.5)	3.748 (t, J=9.5)	3.612 (t, J=10.3)
4-H	3.334 (dd, J=10.3, 9.5)	3.417 (dd, J=9.5, 8.7)	3.515 (dd, J=10.0, 9.5)	3.379 (dd, J=10.3, 9.5)
5-H	1.912 (m)	1.967 (m)	2.097 (m)	2.029 (m)
6-H_{ax}	1.347 (ddd, J=14.6, 12.8, 2.5)	1.384 (ddd, J=14.5, 12.9, 2.5)	1.366 (ddd, J=14.5, 13.0, 2.8)	1.423 (ddd, J=14.5, 13.5, 2.5)
6-H_{eq}	1.978 (dt, J=14.6, 3.3)	1.986 (dt, 14.5, 3.3)	1.960 (dt, J=14.5, 3.2)	2.010 (dt, J=14.5, 2.9)
7-H_a	3.661 (dd, J=11.0, 5.8)	3.702 (dd, J=11.3, 5.5)	3.788 (d, J=4.3, 7-$H_{a,b}$)	3.754 (dd, J=10.3, 5.5)
7-H_b	3.752 (dd, J=11.0, 3.3)	3.749 (dd, J=11.3, 3.3)		4.053 (dd, J=10.3, 2.9)
1′-H	3.433 (br s)	3.460 (br s)	3.382 (br t, J=4.9)	3.391 (br s)
2′-H	6.096 (dq, J=5.0, 1.5)	6,040 (dq, J=5.0, 1.5)	6.046 (dq, J=4.9, 1.5)	6.045 (dq, J=4.5, 1.5)
4′-H	4.069 (br d, J=6.2)	4.104 (br d, J=6.6)	4.095 (br d, J=5.7)	4.097 (br d, J=6.5)
5′-H	3.612 (dd, J=9.9, 6.2)	3.643 (dd, J=10.3, 6.6)	3.633 (dd, J=9.0, 5.7)	3.625 (dd, J=9.5, 6.5)
6′-H	3.649 (dd, J=9.9, 4.5)	3.688 (dd, J=10.3, 5.1)	3.635 (dd, J=9.0, 4.0)	3.645 (dd, J=9.5, 4.5)
7′-H_a	4.135 (br d, J=13.6)	4.149 (br d, J=13.9)	4.138 (br d, J=13.9)	4.137 (br d, J=13.9)
7′-H_b	4.271 (dq, J=13.6, 1.5)	4.255 (br d, J=13.9)	4.251 (dq, J=13.9, 1.5)	4.254 (br d, J=13.9)
1″-H	4.632 (d, J=8.0)	4.652 (d, J=8.0)	4.528 (d, J=8.0)	4.452 (d, J=8.0)
2″-H	3.334 (dd, J=9.2, 8.0)	3.363 (dd, J=9.2, 8.0)	3.347 (dd, J=9.1, 8.0)	3.295 (dd, J=9.2, 8.0)
3″-H	3.504 (t, J=9.2)	3.526 (t, J=9.2)	3.524 (t, J=9.1)	3.502 (t, J=9.2)
4″-H	3.407 (dd, J=9.9, 9.2)	3.408 (dd, J=9.5, 9.2)	3.435 (dd, J=9.5, 9.1)	3.381 (dd, J=9.5, 9.2)
5″-H	3.440 (m)	3.470 (ddd, J=9.5, 5.5, 2.2)	3.497 (m)	3.463 (m)
6″-H_a	3.756 (dd, J=12.5, 5.1)	3.737 (dd, J=12.5, 5.5)	3.744 (dd, J=12.3, 5.8)	3.724 (dd, J=12.5, 5.8)
6″-H_b	3.891 (dd, J=12.5, 1.9)	3.917 (dd, J=12.5, 2.2)	3.916 (dd, J=12.3, 2.1)	3.929 (dd, J=12.5, 2.2)

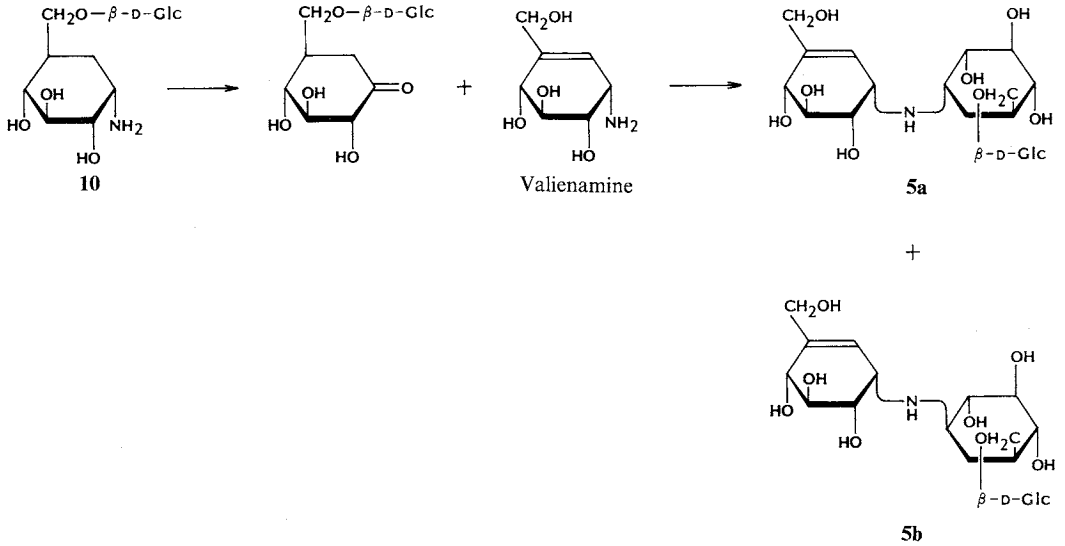
Table 1. (Continued)

Proton	6 (Compound C-IV)	7a	8a	9 (Compound C-I)
1-H	3.289 (br q)	3.295 (br q)	3.288 (br q)	3.289 (br s)
2-H	3.572 (dd, J=10.0, 4.0)	3.577 (dd, J=10.0, 4.5)	3.510 (dd, J=10.0, 4.0)	3.583 (dd, J=10.0, 4.0)
3-H	3.609 (d, J=10.0)	3.594 (t, J=10.0)	3.545 (t, J=10.0)	3.616 (t, J=10.0)
4-H	3.282 (dd, J=10.0, 8.7)	3.287 (dd, J=10.0, 8.7)	3.296 (dd, J=10.0, 8.7)	3.285 (dd, J=10.0, 8.7)
5-H	1.907 (m)	1.911 (m)	1.878 (m)	1.912 (m)
6-H_{ax}	1.326 (ddd, J=14.3, 12.5, 2.9)	1.322 (ddd, J=14.6, 12.1, 2.9)	1.326 (ddd, J=14.7, 12.8, 2.9)	1.321 (ddd, J=14.6, 12.1, 2.5)
6-H_{eq}	1.973 (dt, J=14.3, 3.3)	1.982 (dt, J=14.6, 3.3)	2.076 (dt, J=14.7, 3.3)	1.967 (dt, J=14.6, 3.3)
7-H_a	3.672 (dd, J=11.3, 5.8)	3.672 (dd, J=11.3, 5.8)	3.722 (d, J=4.8, 7-$H_{a,b}$)	3.673 (dd, J=11.2, 5.5)
7-H_b	3.753 (dd, J=11.3, 3.3)	3.745 (dd, J=11.3, 3.4)		3.753 (dd, J=11.2, 3.7)
1′-H	3.414 (br t, J=5.0)	3.469 (br t, J=5.0)	3.710 (br s)	3.409 (br t, J=5.0)
2′-H	6.099 (dq, J=5.0, 1.5)	6.037 (dq, J=5.0, 1.5)	5.952 (dq, J=5.0, 1.5)	6.167 (dq, J=5.0, 1.5)
4′-H	4.262 (br d, J=6.2)	4.245 (br d, J=6.2)	4.120 (br d, J=6.2)	4.151 (br d, J=6.6)
5′-H	3.889 (dd, J=9.5, 6.2)	3.919 (dd, J=9.5, 6.2)	4.065 (dd, J=9.3, 6.2)	3.619 (dd, J=9.5, 6.6)
6′-H	3.729 (dd, J=9.5, 5.0)	3.877 (dd, J=9.5, 5.0)	3.941 (dd, J=9.3, 4.6)	3.669 (dd, J=9.5, 5.0)
7′-H_a	4.184 (br d, J=13.9)	4.138 (br d, J=13.9)	4.140 (br d, J=14.0)	4.361 (br d, J=12.8)
7′-H_b	4.306 (br d, J=13.9)	4.256 (br d, J=13.9)	4.243 (br d, J=14.0)	4.433 (br d, J=12.8)
1″-H	4.656 (d, J=8.0)	4.737 (d, J=8.0)	4.655 (d, J=8.0)	4.506 (d, J=8.0)
2″-H	3.351 (dd, J=9.2, 8.0)	3.362 (dd, J=9.2, 8.0)	3.357 (dd, J=9.2, 8.0)	3.303 (dd, J=9.2, 8.0)
3″-H	3.536 (t, J=9.2)	3.536 (t, J=9.2)	3.518 (t, J=9.2)	3.501 (t, J=9.2)
4″-H	3.429 (dd, J=9.5, 9.2)	3.412 (dd, J=10.0, 9.2)	3.372 (dd, J=10.0, 9.2)	3.386 (dd, J=9.5, 9.2)
5″-H	3.529 (ddd, J=9.5, 6.0, 2.2)	3.508 (ddd, J=10.0, 5.9, 2.2)	3.453 (ddd, J=10.0, 6.1, 2.2)	3.447 (ddd, J=9.5, 5.5, 2.2)
6″-H_a	3.732 (dd, J=12.5, 6.0)	3.728 (dd, J=12.5, 5.9)	3.737 (dd, J=12.6, 6.1)	3.721 (dd, J=12.5, 5.5)
6″-H_b	3.945 (dd, J=12.5, 2.2)	3.938 (dd, J=12.5, 2.2)	3.928 (dd, J=12.6, 2.2)	3.909 (dd, J=12.5, 2.2)

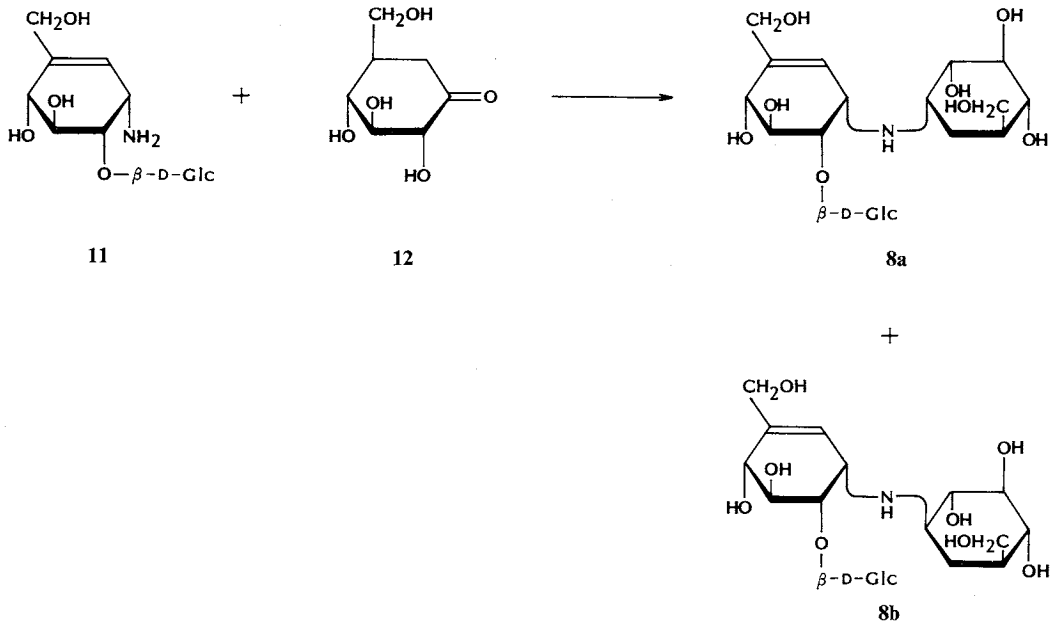
Table 2. ^{13}C NMR data of validoxylamine A and all eight possible mono-β-D-glucosides of validoxylamine A.

Carbon	δ (ppm from TSP)								
	1	2 (Compound C-II)	3a	4 (Compound C-III)	5a	6 (Compound C-IV)	7a	8a	9 (Compound C-I)
C-1	56.7	56.6	57.0	56.5	57.0	56.9	56.8	57.1	57.0
C-2	76.3	86.5	75.5	76.0	76.1	76.2	76.2	76.2	76.1
C-3	77.1	76.5	87.8	75.5	77.1	77.3	77.3	77.6	77.2
C-4	76.2	76.0	74.3	87.1	75.8	76.2	76.2	76.1	76.2
C-5	40.8	40.6	40.8	40.2	39.7	40.9	40.9	40.8	40.9
C-6	29.7	29.5	29.2	29.7	30.1	29.8	29.8	29.5	29.7
C-7	65.3	65.3	65.1	64.6	74.0	65.3	65.3	65.3	65.3
C-1′	55.1	55.0	55.4	55.2	55.3	54.8	55.2	55.6	55.3
C-2′	126.0	126.4	125.3	126.0	126.0	128.2	126.4	126.4	129.2
C-3′	141.9	141.4	142.6	142.0	142.1	139.9	141.4	141.7	139.1
C-4′	74.2	74.0	74.2	74.3	74.3	84.7	72.6	73.9	74.2
C-5′	76.2	76.5	76.4	76.5	76.6	74.9	85.6	74.5	76.4
C-6′	72.1	72.0	72.0	72.2	72.3	71.8	71.6	81.8	72.1
C-7′	64.3	64.6	64.4	64.4	64.4	64.6	64.6	64.6	72.9
C-1″		106.6	106.0	105.7	105.9	106.3	105.8	106.1	104.9
C-2″		76.4	76.4	76.2	76.0	76.3	76.4	76.4	76.0
C-3″		78.5	78.4	78.4	78.5	78.5	78.5	78.5	78.6
C-4″		72.4	72.3	72.2	72.6	72.4	72.5	72.7	72.5
C-5″		78.7	78.9	78.8	78.8	78.9	78.8	78.9	78.8
C-6″		63.5	63.5	63.3	63.6	63.5	63.6	63.8	63.6

Scheme 1.



Scheme 2.

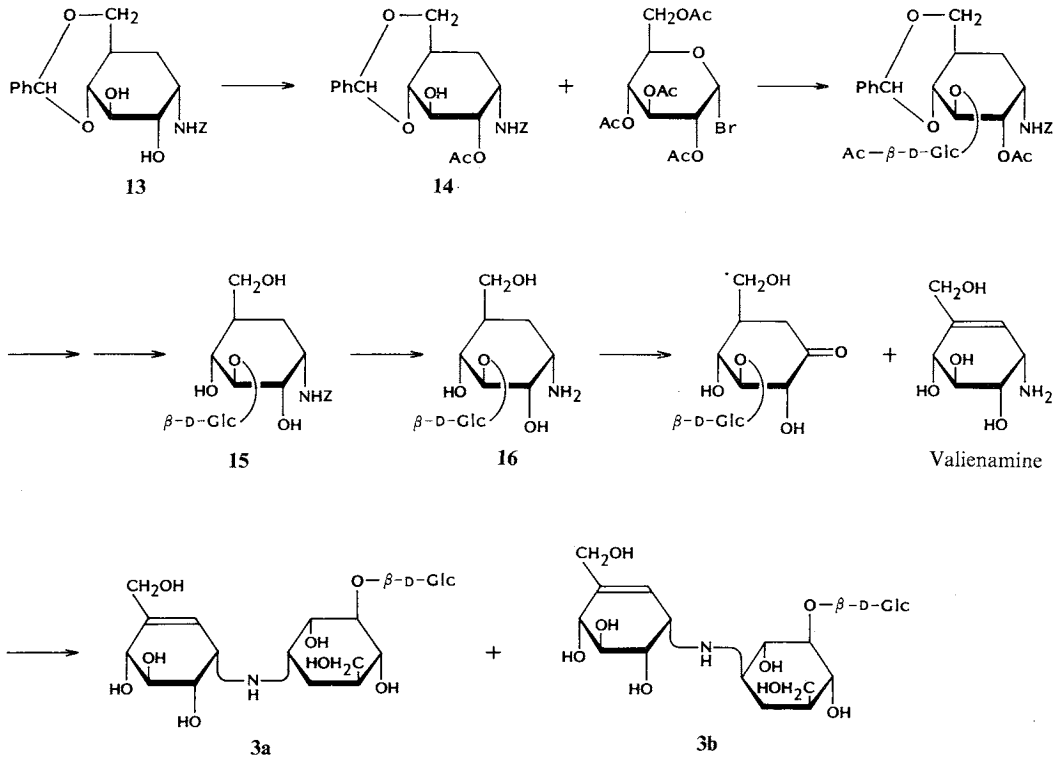


A[5]. From these results, the structure of compound C-IV was shown to be 4′-*O*-β-D-glucopyranosylvalidoxylamine A (**6**).

Semi-synthesis of 7-*O*-β-D-Glucopyranosylvalidoxylamine A (**5a**) and 6′-*O*-β-D-Glucopyranosylvalidoxylamine A (**8a**)

The preparation of 7-*O*-β-D-glucopyranosylvalidamine (**10**), the pseudo-disaccharide unit of **5a** whose decaacetate has been synthesized by OGAWA *et al.*[6], was carried out by microbial glycosylation of validamine with *Rhodotorula lactosa*.

Scheme 3.



The reaction mixture (100 ml) containing validamine (1 g), cellobiose (10 g) and the cell suspension of *R. lactosa* was incubated at 37°C for 48 hours with shaking. By this microbial glycosylation reaction, **10** (105 mg), 2-*O*-*β*-D-glucopyranosylvalidamine and 4-*O*-*β*-D-glucopyranosylvalidamine mixture (520 mg) were obtained. The oxidative deamination of the primary amine of **10** was achieved by treatment with 3,5-di-*tert*-butyl-1,2-benzoquinone[7] under a stream of nitrogen. The coupling of the ketone derivative and valienamine by reductive alkylation with $NaBH_3CN$ gave a mixture of **5a** and its epimer (**5b**) as illustrated in Scheme 1. The stereochemistry at the C-1 position of **5a** was determined by ^1H NMR spectrum δ 3.594 (dd, $J_{1,2}=4.0$ Hz, $J_{2,3}=10.3$ Hz, 2-H) as shown in Table 1.

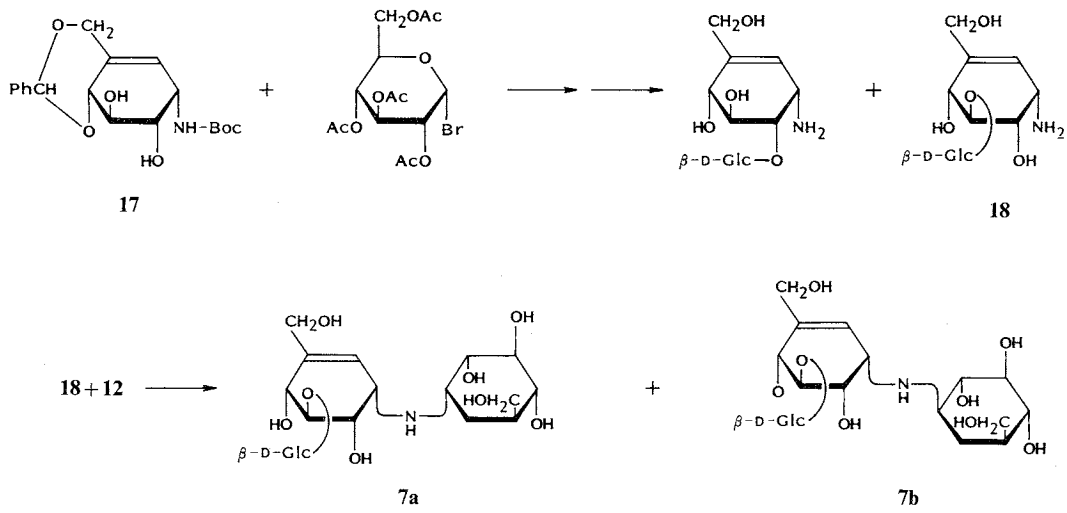
Similarly, 6-*O*-*β*-D-glucopyranosylvalienamine (**11**), the pseudo-disaccharide unit of **8a**, was prepared by microbial glycosylation of valienamine with *R. lactosa*, which has recently been reported by FURUMOTO *et al.*[8].

The preparation of **8a** was carried out by the coupling of **11** with (2*R*)-(2,4/3,5)-2,3,4-trihydroxy-5-hydroxymethylcyclohexanone[7] (**12**) in a similar manner of that described for the preparation of **5a** and then chromatographic separation (Scheme 2). The stereochemistry at the C-1 position of **8a** was determined by ^1H NMR spectrum δ 3.510 (dd, $J_{1,2}=4.0$ Hz, $J_{2,3}=10.0$ Hz, 2-H) as shown in Table 1.

Synthesis of 3-*O*-*β*-D-Glucopyranosylvalidoxylamine A (**3a**) and 5′-*O*-*β*-D-Glucopyranosylvalidoxylamine A (**7a**)

3-*O*-*β*-D-Glucopyranosylvalidoxylamine A (**3a**) was synthesized as illustrated in Scheme 3. The pseudo-disaccharide unit of **3a**, 3-*O*-*β*-D-glucopyranosylvalidamine (**16**), was prepared by condensation of a suitably protected validamine derivative (**14**) with acetobromoglucose, and successive removal of the

Scheme 4.



protecting groups.

Synthesis of **16** has been reported by SUAMI *et al.*[9,10] and led to revision of the proposed structure[11] of validamycin A. The hydroxy group at the C-2 position of 4,7-*O*-benzylidene-*N*-(benzyloxycarbonyl)-validamine[12] (**13**) was selectively acetylated with *N*-acetylimidazole in dimethyl sulfoxide-pyridine (9:1) according to the reported method[13]. The condensation of **14** with acetobromoglucose was conducted in the presence of silver trifluoromethanesulfonate (AgOTf) and 1,1,3,3-tetramethylurea (TMU) in dry dichloromethane at room temperature for 16 hours. Removal of the benzylidene and acetyl groups of the resulting β-glucoside, followed by chromatographic separation with Amberlite XAD-4 and Toyopearl HW-40S, gave 3-*O*-β-D-glucopyranosyl-*N*-(benzyloxycarbonyl)validamine (**15**). Catalytic hydrogenolysis of **15** over palladium on carbon removed the benzyloxycarbonyl group to give **16**. The oxidative deamination of **16** with 3,5-di-*tert*-butyl-1,2-benzoquinone, and coupling of the resulting ketone derivative and valienamine by reductive alkylation with $NaBH_3CN$ gave a mixture of **3a** and its 1-epimer (**3b**). Both epimers were separated by column chromatography. The stereochemistry at the C-1 position of **3a** was determined by ^1H NMR spectrum δ 3.797 (dd, $J_{1,2}=4.0$ Hz, $J_{2,3}=9.5$ Hz, 2-H) as shown in Table 1.

5'-*O*-β-D-Glucopyranosylvalidoxylamine A (**7a**), which is the last one of eight theoretically possible mono-β-D-glucosides of **1**, was synthesized as illustrated in Scheme 4. The starting material, *N*-(*tert*-butoxycarbonyl)valienamine[14] was converted into the 4,7-*O*-benzylidene derivative (**17**) by treatment with benzaldehyde dimethyl acetal and *p*-toluenesufonic acid in *N*,*N*-dimethylformamide. Selective acetylation of **17** could not be achieved; treatment of **17** with *N*-acetylimidazole in dimethyl sulfoxide-pyridine (9:1) afforded the 5,6-di-*O*-acetyl derivative. Therefore, we carried out a condensation reaction of the 5,6-diol (**17**) with the halide. The condensation of **17** with acetobromoglucose in the presence of AgOTf and TMU in dry dichloromethane at room temperature for 48 hours gave a mixture of products. The resulting two β-D-glucosides were inseparable by column chromatography on silica gel. Removal of the protecting groups, followed by chromatographic separation, gave 5-*O*-β-D-glucopyranosylvalienamine (**18**) and 6-*O*-β-D-glucopyranosylvalienamine with the ratio of 4:1. Coupling of **18** and **12** by reductive alkylation with $NaBH_3CN$ gave a mixture of **7a** and its 1-epimer

(**7b**). Both epimers were separated by column chromatography. The stereochemistry at the C-1 position of **7a** was determined by ^{1}H NMR spectrum δ 3.577 (dd, $J_{1,2}=4.5$ Hz, $J_{2,3}=10.0$ Hz, 2-H) as shown in Table 1.

Experimental

MP's were determined with a Yamato MP-21 and are uncorrected. Optical rotations were measured with a Jasco DIP-370 digital polarimeter. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded with a Jeol JNM-GX 400 spectrometer in deuterium oxide (D_2O) containing sodium 3-(trimethylsilyl)propionate (TSP) as an internal standard. Mass spectra were recorded with a Jeol JMS-DX300 JMA-DA5000 spectrometer. TLC was performed on HPTLC Silica gel 60 F_{254} (Merck) using solvent system I (PrOH - AcOH - H_2O, 4:1:1) or II (BuOH - AcOH - H_2O, 6:3:1). HPLC analysis was performed on a YMC PA-5 column (4.6×150 mm) eluted at 3 ml/minute flow rate with CH_3CN - H_2O (80:20) as a mobile phase and elution was followed by UV detection at 210 nm.

Microbial β-Glycosylation of Validamine

R. lactosa IFO 1424 was cultured with shaking at 37°C for 48 hours in a medium 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 10,000×*g* for 15 minutes, washed with 10 mM phosphate buffer (pH 6.0). The washed cells (15 g, wet weight) were suspended in 50 mM phosphate buffer (pH 6.0, 100 ml). The cell suspensions containing 1 g of validamine and 10 g of cellobiose were incubated at 37°C for 48 hours with shaking. The incubation mixture was centrifuged and the supernatant was applied to a column of Dowex 50W-X2 (H^+, 100 ml) and eluted with 0.5 N NH_4OH. The concentrate was applied to a column of Dowex 1-X2 (OH^-, 2.5×90 cm) and eluted with water to give 7-*O*-β-D-glucopyranosylvalidamine (**10**, 105 mg), and 2-*O*-β-D-glucopyranosylvalidamine and 4-*O*-β-D-glucopyranosylvalidamine mixture (520 mg).

10: Rf 0.26 (System I); $[\alpha]_D^{25}$ +16.7° (*c* 1, H_2O); ^{13}C NMR (D_2O) δ 32.1 (t, C-6), 39.6 (d, C-5), 52.8 (d, C-1), 63.7 (t, C-6'), 72.7 (d, C-4'), 74.1 (t, C-7), 75.9 (d, C-4), 76.1 (d, C-2, C-2'), 76.9 (d, C-3), 78.7 (d, C-3'), 78.9 (d, C-5'), 106.1 (d, C-1').

The glucoside mixture (520 mg) and $NaHCO_3$ (500 mg) were dissolved in water (5 ml), and a solution of benzyloxycarbonyl chloride (0.8 ml) in toluene (2.5 ml) was added dropwise to the solution under ice cooling, followed by stirring at the same temperature for 1 hour and then at room temperature for 3 hours. The reaction mixture was adjusted to pH 5 with 2 N HCl and extracted with toluene. The water layer was applied to a column of Amberlite XAD-4 (20 ml), eluted with MeOH and concentrated. The concentrate was then applied to a column of Toyopearl HW-40S (2.2×115 cm) and eluted with 20% MeOH to give 2-*O*-β-D-glucopyranosyl-*N*-(benzyloxycarbonyl)validamine (340 mg) and 4-*O*-β-D-glucopyranosyl-*N*-(benzyloxycarbonyl)validamine (210 mg) in order of elution from the column.

2-*O*-β-D-Glucopyranosyl-*N*-(benzyloxycarbonyl)validamine: Rf 0.44 (System II); $[\alpha]_D^{25}$ +44.9° (*c* 1, H_2O); ^{13}C NMR (D_2O) δ 31.5 (t, C-6), 41.1 (d, C-5), 53.3 (d, C-1), 63.8 (t, C-6'), 64.9 (t, C-7), 69.8 (t, Ph–CH_2–), 72.5 (d, C-4'), 75.5 (d, C-4), 76.3 (d, C-2'), 76.6 (d, C-3), 78.4 (d, C-3'), 78.7 (d, C-5'), 84.9 (d, C-2), 106.8 (d, C-1'), 130.6, 131.3, 131.7, 139.6, (C_6H_5–), 161.4 (s, –NH–COO–).

4-*O*-β-D-Glucopyranosyl-*N*-(benzyloxycarbonyl)validamine: Rf 0.45 (System II); $[\alpha]_D^{25}$ +36.4° (*c* 1, H_2O); ^{13}C NMR (D_2O) δ 31.3 (t, C-6), 40.9 (d, C-5), 53.4 (d, C-1), 63.4 (t, C-6'), 64.3 (t, C-7), 69.9 (t, Ph–CH_2–), 72.3 (d, C-4'), 74.9 (d, C-3), 76.0 (d, C-2), 76.3 (d, C-2'), 78.5 (d, C-3'), 78.9 (d, C-5'), 86.5 (d, C-4), 105.8 (d, C-1'), 130.7, 131.3, 131.7, 139.5 (C_6H_5–), 161.4 (s, –NH–COO–).

A solution of 2-*O*-β-D-glucopyranosyl-*N*-(benzyloxycarbonyl)validamine (340 mg) in 50% EtOH (50 ml) and AcOH (10 ml) was hydrogenated in the presence of 5% palladium on carbon (1.2 g) at atmospheric pressure for 24 hours at room temperature. The mixture was processed conventionally and purified by Dowex 1-X2 (OH^-, 50 ml) chromatography with water to give 2-*O*-β-D-glucopyranosylvalidamine (227 mg). 4-*O*-β-D-Glucopyranosyl-*N*-(benzyloxycarbonyl)validamine (210 mg) was also treated by the same procedures as described above to give 4-*O*-β-D-glucopyranosylvalidamine (145 mg).

2-*O*-β-D-Glucopyranosylvalidamine: Rf 0.30 (System I); $[\alpha]_D^{25}$ +27.1° (*c* 1, H_2O); ^{13}C NMR (D_2O) δ 32.0 (t, C-6), 40.6 (d, C-5), 51.7 (d, C-1), 63.4 (t, C-6'), 65.4 (t, C-7), 72.4 (d, C-4'), 76.1 (d, C-4), 76.2

(d, C-2'), 76.3 (d, C-3), 78.5 (d, C-3'), 78.7 (d, C-5'), 87.3 (d, C-2), 106.5 (d, C-1').

4-*O*-*β*-D-Glucopyranosylvalidamine: Rf 0.28 (System I); $[\alpha]_D^{25}$ +30.5° (*c* 1, H_2O); ^{13}C NMR (D_2O) δ 32.3 (t, C-6), 40.3 (d, C-5), 52.2 (d, C-1), 63.4 (t, C-6'), 64.7 (t, C-7), 72.3 (d, C-4'), 75.4 (d, C-3), 76.4 (d, C-2, C-2'), 78.6 (d, C-3'), 78.9 (d, C-5'), 87.1 (d, C-4), 105.9 (d, C-1').

Microbial *β*-Glycosylation of Valienamine

The cell suspensions containing 1 g of valienamine and 10 g of cellobiose were incubated at 37°C for 24 hours with shaking. The incubation mixture was treated in a similar manner of that described for transglucosylation of validamine. The transglucosylation products were purified on a column of Dowex 1-X2 (OH^-, 2.5×90 cm) and eluted with water to give 7-*O*-*β*-D-glucopyranosylvalienamine (130 mg), 6-*O*-*β*-D-glucopyranoyslvalienamine (**11**, 190 mg) and 4-*O*-*β*-D-glucopyranosylvalienamine (60 mg) in order of elution from the column. Physico-chemical properties and NMR data of these compounds are shown in the literature[8].

7-*O*-*β*-D-Glucopyranosylvalidoxylamine A (**5a**)

10 (100 mg) and 3,5-di-*tert*-butyl-1,2-benzoquinone (95 mg) were dissolved in MeOH (5 ml), and the solution was stirred under a stream of nitrogen at room temperature for 24 hours. After adding water (1 ml), the reaction mixture was adjusted to pH 1.2 with 2 N H_2SO_4 and stirred at room temperature for 3 hours. After adding water (25 ml), the reaction mixture was washed five times with chloroform. The water layer is adjusted to pH 5.5 with Dowex 1-X2 (OH^-) resin. The reaction mixture was filtered and concentrated under reduced pressure. The residue (45 mg) and valienamine (60 mg) were dissolved in DMF (3 ml), and 2 N HCl (0.12 ml) and $NaBH_3CN$ (150 mg) were added to the solution, followed by stirring at 37°C for 17 hours. The reaction mixture was concentrated under reduced pressure, and the residue was dissolved in water (10 ml) and chromatographed on a column of Dowex 50W-X2 (H^+, 10 ml). The column was washed with water and eluted with 0.5 N NH_4OH. The eluate was concentrated and applied to a column of Amberlite CG-50 (NH_4^+, 8 ml). The effluent and washings were concentrated and then applied to a column of Dowex 1-X2 (OH^-, 1×35 cm). The column was eluted with water to give 7-*O*-*β*-D-glucopyranosylvalidoxylamine A (**5a**, 19 mg) and (1*R*)-(1,3,5/2,4)-5-*O*-(*β*-D-glucopyranosyloxymethyl)-2,3,4-trihydroxy-*N*-[(1*S*)-(1,4,6/5)-3-hydroxymethyl-4,5,6-trihydroxycyclohex-2-enyl]cyclohexylamine (**5b**, 5 mg), respectively.

5a: Rf 0.29 (System I); HPLC Rt 12.0 minutes; $[\alpha]_D^{25}$ +84.6° (*c* 1, H_2O); NMR, see Tables 1 and 2.

5b: Rf 0.28 (System I); Rt 18.5 minutes; $[\alpha]_D^{25}$ +32.6° (*c* 0.8, H_2O); ^{13}C NMR (D_2O) δ 33.2 (t, C-6), 42.9 (d, C-5), 57.1 (d, C-1'), 62.3 (d, C-1), 63.8 (t, C-6''), 64.6 (t, C-7'), 71.8 (d, C-6'), 72.7 (d, C-4''), 73.8 (d, C-4'), 73.9 (t, C-7), 75.2 (d, C-4), 75.7 (d, C-2''), 76.2 (d, C-5'), 78.7 (d, C-3''), 78.8 (C-2), 78.9 (d, C-5''), 80.5 (d, C-3), 106.1 (d, C-1''), 127.4 (d, C-2'), 142.1 (s, C-3').

6'-*O*-*β*-D-Glucopyranosylvalidoxylamine A (**8a**)

11 (60 mg) and (2*R*)-(2,4/3,5)-trihydroxy-5-hydroxymethylcyclohexanone[7] (**12**, 100 mg) were dissolved in DMF (3 ml), and 2 N HCl (0.12 ml) and $NaBH_3CN$ (150 mg) were added to the solution, followed by stirring at 27°C for 24 hours. The reaction mixture was treated in a similar manner to that described for the preparation of **5a**. A mixture of coupling products was finally applied to a column of Dowex 1-X2 (OH^-, 1×35 cm) and eluted with water to give 6'-*O*-*β*-D-glucopyranosylvalidoxylamine A (**8a**, 42 mg) and (1*R*)-(1,3,5/2,4)-2,3,4-trihydroxy-5-hydroxymethyl-*N*-[(1*S*)-(1,4,6/5)-6-*O*-(*β*-D-glucopyranosyl)-3-hydroxymethyl-4,5-dihydroxycyclohex-2-enyl]cyclohexylamine (**8b**, 14 mg), respectively.

8a: Rf 0.31 (System I); Rt 12.0 minutes; $[\alpha]_D^{25}$ +81.8° (*c* 1, H_2O); NMR, see Tables 1 and 2.

8b: Rf 0.31 (System I); Rt 14.9 minutes; $[\alpha]_D^{25}$ +21.1° (*c* 1, H_2O); ^{13}C NMR (D_2O) δ 32.2 (t, C-6), 44.0 (d, C-5), 55.1 (d, C-1'), 60.3 (d, C-1), 63.4 (t, C-6''), 64.8 (t, C-7'), 65.3 (t, C-7), 72.3 (d, C-4''), 72.9 (d, C-4'), 73.6 (d, C-5'), 75.7 (d, C-4), 76.4 (d, C-2''), 78.4 (d, C-3''), 78.6 (d, C-2), 79.0 (d, C-5''), 80.0 (d, C-6'), 80.5 (d, C-3), 105.6 (d, C-1''), 128.1 (d, C-2'), 141.4 (s, C-3').

3-*O*-*β*-D-Glucopyranosylvalidoxylamine A (**3a**)

A suspension of 4,7-*O*-benzylidene-*N*-(benzyloxycarbonyl)validamine[12] (**13**, 4.8 g) and *N*-acetylimidazole (4.0 g) in DMSO (45 ml) and pyridine (5 ml) was stirred for 48 hours at room temperature.

The resulting solution was concentrated under reduced pressure and the residual syrup was dissolved in EtOAc. The solution was washed with 2 N HCl and saturated aqueous $NaHCO_3$, and dried over $MgSO_4$. The filtrate was concentrated and chromatographed on a column of silica gel (200 ml) eluted with hexane - EtOAc (1 : 1) to give 2-*O*-acetyl-4,7-*O*-benzylidene-*N*-(benzyloxycarbonyl)validamine (**14**, 1.2 g).

14: MP 193~195°C (dec); $[\alpha]_D^{25}$ +47.0° (*c* 1, $CHCl_3$); EI-MS *m/z* 441 (M^+).

Anal Calcd for $C_{24}H_{27}NO_7$: C 65.29, H 6.16, N 3.17.
Found: C 65.11, H 5.99, N 3.09.

To a stirred solution of **14** (660 mg) in dry CH_2Cl_2 (5 ml) were added AgOTf (670 mg), TMU (0.7 ml) and molecular sieves 4A (1 g), and then acetobromoglucose (1.2 g) was added to the mixture. The reaction mixture was stirred at room temperature for 16 hours, diluted with CH_2Cl_2 (15 ml) and filtered through Celite. The filtrate was concentrated and the residue dissolved in a mixture of concd NH_4OH - MeOH (1 : 4, 40 ml), followed by stirring overnight at room temperature. The reaction mixture was concentrated and the residue was dissolved in MeOH (33 ml). To the solution was added 0.5 N HCl (10 ml), followed by stirring under reflux for 30 minutes. The reaction mixture was cooled to room temperature, adjusted to pH 5.0 with saturated aqueous $NaHCO_3$ and concentrated under reduced pressure. The residue was dissolved in water, applied to a column of Amberlite XAD-4 (20 ml) and eluted with MeOH. The eluate was concentrated and then applied to a column of Toyopearl HW-40S (2.2 × 115 cm). The column was eluted with 20% MeOH to give 3-*O*-*β*-D-glucopyranosyl-*N*-(benzyloxycarbonyl)validamine (**15**, 380 mg).

15: Rf 0.53 (System II); $[\alpha]_D^{25}$ +22.7° (*c* 1, H_2O); ^{13}C NMR (D_2O) *δ* 31.2 (t, C-6), 41.3 (d, C-5), 53.7 (d, C-1), 63.6 (t, C-6′), 64.9 (t, C-7), 69.9 (t, Ph–CH_2–), 72.4 (d, C-4′), 74.1 (d, C-4), 74.8 (d, C-2), 76.5 (d, C-2″), 78.5 (d, C-3′), 79.0 (d, C-5′), 87.6 (d, C-3), 106.0 (d, C-1′), 130.7, 131.3, 131.7, 139.4 (C_6H_5–), 161.4 (s, –NH–COO–).

A solution of **15** (320 mg) in 50% EtOH and AcOH (10 ml) was hydrogenated in the presence of 5% palladium on carbon (1 g) at atmospheric pressure for 24 hours at room temperature. The mixture was processed conventionally and purified by Dowex 1-X2 (OH^-, 50 ml) chromatography with water to give 3-*O*-*β*-D-glucopyranosylvalidamine (**16**, 210 mg).

16: Rf 0.34 (System I); $[\alpha]_D^{25}$ +28.4° (*c* 1, H_2O); ^{13}C NMR (D_2O) *δ* 31.4 (t, C-6), 40.7 (d, C-5), 52.8 (d, C-1), 63.6 (t, C-6′), 65.1 (t, C-7), 72.4 (d, C-4′), 74.3 (d, C-4), 75.5 (d, C-2), 76.5 (d, C-2′), 78.5 (d, C-3′), 79.0 (d, C-5′), 87.6 (d, C-3), 106.2 (d, C-1′).

16 (200 mg) was deaminated oxidatively with 3,5-di-*tert*-butyl-1,2-benzoquinone in a similar manner to that described for the preparation of **5a**. The resulting ketone derivative (154 mg) and valienamine (150 mg) were dissolved in DMF (5 ml), and 2 N HCl (0.2 ml) and $NaBH_3CN$ (200 mg) were added to the solution, followed by stirring at room temperature for 24 hours. The reaction mixture was concentrated under reduced pressure, and the residue dissolved in water (10 ml) and chromatographed on a column of Dowex 50W-X2 (H^+, 15 ml). The column was washed with water and eluted with 0.5 N NH_4OH. The eluate was concentrated and applied to a column of Amberlite CG-50 (NH_4^+, 15 ml). The effluent and washings were concentrated and then applied to a column of Dowex 1-X2 (OH^-, 1.5 × 15 cm) equilibrated with 50% MeOH. The column was eluted with 50% MeOH to give 3-*O*-*β*-D-glucopyranosylvalidoxylamine A (**3a**, 45 mg) and (1*R*)-(1,3,5/2,4)-3-*O*-(*β*-D-glucopyranosyl)-2,4-dihydroxy-5-hydroxymethyl-*N*-[(1*S*)-(1,4,6/5)-3-hydroxymethyl-4,5,6-trihydroxycyclohex-2-enyl]cyclohexylamine (**3b**, 7 mg), respectively.

3a: Rf 0.36 (System I); Rt 7.3 minutes; $[\alpha]_D^{25}$ +93.7° (*c* 1, H_2O); NMR, see Tables 1 and 2.

3b: Rf 0.34 (System I); Rt 11.9 minutes; $[\alpha]_D^{25}$ +25.5° (*c* 1, H_2O); ^{13}C NMR (D_2O) *δ* 33.0 (t, C-6), 43.7 (d, C-5), 56.1 (d, C-1′), 62.0 (d, C-1), 63.6 (t, C-6″), 64.6 (t, C-7′), 65.2 (t, C-7), 72.0 (d, C-6′), 72.4 (d, C-4″), 73.9 (d, C-4′), 74.1 (d, C-4), 75.8 (d, C-2″), 76.5 (d, C-5′), 78.6 (d, C-2, C-3″), 78.9 (d, C-5″), 90.2 (d, C-3), 106.1 (d, C-1″), 128.0 (d, C-2′), 141.6 (s, C-3′).

5′-*O*-*β*-D-Glucopyranosylvalidoxylamine A (**7a**)

A mixture of *N*-(*tert*-butoxycarbonyl)valienamine[14] (2 g), benzaldehyde dimethyl acetal (1.1 g) and *p*-toluenesulfonic acid (5 mg) in DMF (10 ml) was stirred at 60~65°C for 1 hour under 60 mmHg and then concentrated under reduced pressure. The residual syrup was dissolved in EtOAc. The solution was washed with aqueous $NaHCO_3$. After removal of organic solvent, Et_2O (150 ml) was added to the residue and then the mixture was refrigerated to give 4,7-*O*-benzylidene-*N*-(*tert*-butoxycarbonyl)valienamine

(**17**, 2.2 g).

17: $[\alpha]_D^{25}$ +75.5° (*c* 1, $CHCl_3$); EI-MS *m/z* 364 (M+H).

Anal Calcd for $C_{19}H_{25}NO_6 \cdot H_2O$: C 59.83, H 7.13, N 3.67.
Found: C 60.05, H 6.97, N 3.81.

To a stirred solution of **17** (2 g) in dry CH_2Cl_2 (20 ml) were added AgOTf (2.4 g), TMU (2.8 ml) and molecular sieves 4A (3 g), and then acetobromoglucose (3.6 g) was added to the mixture. The reaction mixture was stirred at room temperature for 48 hours with CH_2Cl_2 (50 ml), and filtered through Celite. The filtrate was concentrated and the residue was dissolved in a mixture of concd NH_4OH - MeOH (1 : 4, 80 ml), followed by stirring overnight at room temperature. The reaction mixture was concentrated and the residue was dissolved in MeOH (100 ml). To the solution was added 0.5 N HCl (30 ml), followed by stirring under reflux for 15 minutes. The reaction mixture was cooled to room temperature, adjusted to pH 5.0 with saturated aqueous $NaHCO_3$ and concentrated under reduced pressure. The residue was dissolved in water, applied to a column of Amberlite CG-50 (NH_4^+, 100 ml) and eluted with 0.1 N NH_4OH. The glucoside mixture (480 mg) was then applied to a column of Dowex 1-X2 (2.5×90 cm) and eluted with water to give 5-*O*-*β*-D-glucopyranosylvalienamine (**18**, 280 mg) and 6-*O*-*β*-D-glucopyranosylvalienamine (75 mg), respectively.

18: Rf 0.37 (System I); $[\alpha]_D^{25}$ +41.2° (*c* 1, H_2O); ^{13}C NMR (D_2O) δ 51.7 (d, C-1), 63.7 (t, C-6′), 64.4 (t, C-7), 71.6 (d, C-6), 72.6 (d, C-4′), 72.8 (d, C-4), 76.4 (d, C-2′), 78.6 (d, C-3′), 79.0 (d, C-5′), 84.9 (d, C-5), 106.0 (d, C-1′), 126.0 (d, C-2), 142.8 (s, C-3).

18 (100 mg) and **12** (150 mg) were dissolved in DMF (5 ml), and 2 N HCl (0.2 ml) and $NaBH_3CN$ (200 mg) were added to the solution, followed by stirring at 50~60°C for 17 hours. The reaction mixture was treated in a similar manner to that described for the preparation of **5a**. A mixture of coupling products was finally applied to a column of Dowex 1-X2 (OH^-, 1.5×33 cm) and eluted with water to give 5′-*O*-*β*-D-glucopyranosylvalidoxylamine A (**7a**, 90 mg) and (1*R*)-(1,3,5/2,4)-2,3,4-trihydroxy-5-hydroxymethyl-*N*-[(1*S*)-(1,4,6/5)-5-*O*-(*β*-D-glucopyranosyl)-3-hydroxymethyl-4,6-dihydroxycyclohex-2-enyl]cyclohexylamine (**7b**, 17 mg), respectively.

7a: Rf 0.33 (System I); Rt 8.5 minutes; $[\alpha]_D^{25}$ +89.5° (*c* 1, H_2O); NMR, see Tables 1 and 2.

7b: Rf 0.34 (System I); Rt 12.2 minutes; $[\alpha]_D^{25}$ +20.6° (*c* 1, H_2O); ^{13}C NMR (D_2O) δ 32.7 (t, C-6), 44.0 (d, C-5), 55.9 (d, C-1′), 61.0 (d, C-1), 63.7 (t, C-6″), 64.8 (t, C-7′), 65.3 (t, C-7), 70.0 (d, C-6′), 71.8 (d, C-4′), 72.6 (d, C-4″), 75.7 (d, C-4), 76.3 (d, C-2″), 78.6 (d, C-3″), 78.9 (d, C-2, C-5″), 80.5 (d, C-3), 84.6 (d, C-5′), 105.9 (d, C-1″), 128.1 (d, C-2′), 140.9 (s, C-3′).

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References

1) IWASA, T.; H. YAMAMOTO & M. SHIBATA: Studies on validamycins, new antibiotics. I. *Streptomyces hygroscopicus* var. *limoneus* nov. var., validamycin-producing organism. J. Antibiotics 23: 595~602, 1970
2) KAMEDA, Y.; N. ASANO, O. WAKAE & T. IWASA: Microbial glycosidation of validamycins. II. The preparation of α and *β*-D-glucoside analogs of validamycins. J. Antibiotics 33: 764~766, 1980
3) IWASA, T.; E. HIGASHIDE & M. SHIBATA: Studies on validamycins, new antibiotics. III. Bioassay methods for the determination of validamycin. J. Antibiotics 24: 114~118, 1971
4) YAMASAKI, K.; H. KOHDA, T. KOBAYASHI, R. KASAI & O. TANAKA: Structures of Stevia diterpene-glucosides: application of ^{13}C NMR. Tetrahedron Lett. 1976: 1005~1008, 1976
5) YOKOSE, K.; K. OGAWA, Y. SUZUKI, I. UMEDA & Y. SUHARA: New α-amylase inhibitor, trestatins. II. Structure determination of trestatins A, B and C. J. Antibiotics 36: 1166~1175, 1983
6) OGAWA, S.; T. NOSE, T. OGAWA, T. TOYOKUNI, Y. IWASAWA & T. SUAMI: Synthetic studies on antibiotic validamycins. Part 11. Synthesis of validamycin A. J. Chem. Soc. Perkin Trans. I 1985: 2369~2374, 1985
7) HORII, S.; Y. KAMEDA & H. FUKASE (Takeda Chemical Ind.): *N*-Substituted pseudo-aminosugars, and their use. Eur. Pat. Appl. 89 812, 1983 [CA 101: 38779c, 1984]
8) FURUMOTO, T.; T. YOSHIOKA, K. KAMATA, Y. KAMEDA & K. MATSUI: Enzymic synthesis of valienamine glucosides

and their antibiotic activity. J. Antibiotics 44: 371～373, 1991

9) SUAMI, T.; S. OGAWA & N. CHIDA: The revised structure of validamycin A. J. Antibiotics 33: 98～99, 1980

10) OGAWA, S.; Y. SHIBATA, N. CHIDA & T. SUAMI: Synthesis of β-D-glucopyranosylvalidamine: 2-*O*-β-D-glucopyranosyl-1L-(1,3,4/2,6)-4-amino-6-hydroxymethyl-1,2,3-cyclohexanetriol. Chemistry Lett. 1980: 135～138, 1980

11) HORII, S. & Y. KAMEDA: Structure of the antibiotic validamycin A. J. Chem. Soc. Chem. Commun. 1972: 747～748, 1972

12) HORII, S.; H. FUKASE, T. MATSUO, Y. KAMEDA, N. ASANO & K. MATSUI: Synthesis and α-D-glucosidase inhibitory activity of *N*-substituted valiolamine derivatives as potential oral antidiabetic agents. J. Med. Chem. 29: 1038～1046, 1986

13) UMEZAWA, H.; S. UMEZAWA, O. TSUCHIYA & E. UMEMURA (Microbiochemical Research Foundation): 3′-Fluoro-3′-deoxykanamycin A. Jpn. Kokai 140596 ('86), June 27, 1986 [CA 106: 5373r, 1987]

14) HORII, S.; H. FUKASE & Y. KAMEDA: Stereoselective conversion of valienamine and validamine into valiolamine. Carbohydr. Res. 140: 185～200, 1985