MICROBIAL TRANSFORMATION OF VALIDAMYCINS

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(Received for publication January 18, 1975)

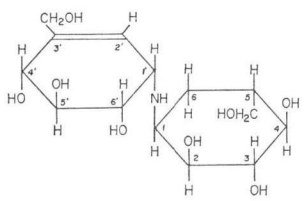
α- or β-Glucosidic linkage of validamycin was selectively cleaved by microbial hydrolysis, and especially the conversion of validamycin C into validamycin A by the selective hydrolysis of α-glucosidic linkage has important significance because validamycin C is considerably less active than validamycin A. Semisyntheses of validamycins including a new validamycin, β-D-galactosyl-validoxylamine A were carried out by microbial transglycosidation using validoxylamine A as a glycosyl acceptor. D-[U-^{14}C]glucose and [^{14}C]validoxylamine A were highly incorporated into validamycin A by validamycin-producing *Streptomyces hygroscopicus* var. *limoneus*.

Streptomyces hygroscopicus var. *limoneus* is known to produce validamycins A~F, validoxylamines A and B.[1~8] The chemical structure of validamycin A[10] and the structure-activity relationship[8] of validamycin group antibiotics were reported in previous papers. Validamycins A, C, D, E* and F contain validoxylamine A[5,10] as common moiety in their molecules, but differ from one another at least in one of the following characteristics; the configuration of anomeric center of glucoside, the position of glucosidic linkage and the number of D-glucose molecules.

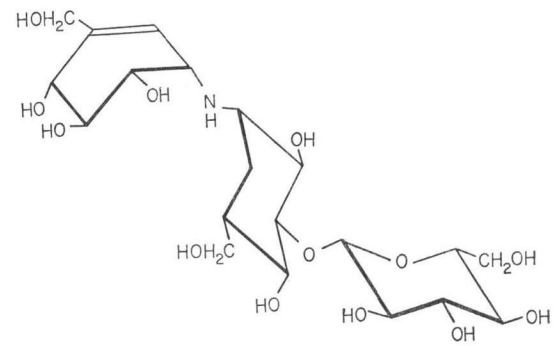
Validamycins have been shown to be susceptible to microbial attack and their addition to soil has resulted in a complete loss of biological activity by soil microbes.[11] Microbial degradation of validamycin A by *Pseudomonas denitrificans* was reported in the previous report.[9] The microbial hydrolysis by *Ps. denitrificans* is non-specific for α- and β-glucosidic linkages, and both validamycins A and D are hydrolyzed to D-glucose and validoxylamine A in the first step. The further decomposition of validoxylamine A proceeds *via* validamine[6,9] and valienamine[9] which can be isolated as the intermediate products.

Microbial transformations of many antibiotics[12,13] including hydrolytic cleavages and prepara-

Validoxylamine A



Structure of validamycin A



* The structure of validamycin E has been established to be 3-O-(4-O-α-D-glucopyranosyl-β-D-glucopyranosyl)-validoxylamine A.

tions of a glycoside of aminoglycoside antibiotics such as mannosidostreptomycin,[14] neamine[15] and kanamycin[16] as substrates, were reported.

A large number of the microorganisms was subjected to the screening of strains capable of hydrolyzing selectively α- or β-glucoside linkage of validamycins A and D. Validamycins C, E and F were transformed into validamycin A by the selective microbial hydrolysis of an α-glucosidic linkage, and validamycins A, D and E were transformed into validoxylamine A by the selective hydrolysis of a β-glucosidic linkage.

In the incorporation experiments of D-[U-^{14}C]glucose and [^{14}C]validoxylamine A using the validamycin-producing *Streptomyces*,[1] both of them were highly incorporated to validamycin A. When D-[U-^{14}C]glucose was added, approximately uniform distribution of radioactivity was observed in each of three moieties (validamine, valienamine and D-glucose) of validamycin A molecule, except that the distribution slightly varied with the culture phase of D-[U-^{14}C]glucose addition as shown in Tables 1 and 2.

Table 1. Distribution of radioactivity in [^{14}C] validamycin A produced from D-[U-^{14}C]glucose. D-[U-^{14}C]glucose was added after 5 days from inoculation

Substance	Specific radioactivity	
	μCi/g	μCi/mmol
Validamycin A	36.00	18.56
D-Glucose	32.40	5.84
Validoxylamine A	34.40	12.16
Validamine	31.32	5.55
Valienamine	35.22	6.17

When [^{14}C]validoxylamine A was added, practically all radioactivity was found in the validoxylamine A moiety and practically no incorporation into D-glucose moiety was shown. As these facts offered a possibility for the semisyntheses of validamycins by the transglycosidation reaction using validoxylamine A as an acceptor, the screening of the microorganisms having transglycosidation activity was carried out. The glucosidation of validoxylamine A into validamycins A and D was consequently achieved by several microorganisms, and β-D-galactopyranosyl-validoxylamine A, a new validamycin was also synthesized.

Table 2. Effect of the culture age of D-[U-^{14}C]glucose addition on the distribution of radioactivity in validamycin A

Time of addition	1 Day			5 Days		
Substance	Total activity	Specific activity		Total activity	Specific activity	
	(μCi)	(μCi/g)	(μCi/mol/Cn*)	(μCi)	(μCi/g)	(μCi/mol/Cn*)
Validamycin A	0.373	0.775	19.98	0.569	1.160	29.90
Validoxylamine A	0.245	0.870	21.96	0.336	1.121	28.30
D-Glucose	0.108	0.646	19.40	0.162	1.123	33.72

* Cn: The number of carbon atoms in a molecule.

Materials and Methods

Screening test for obtaining microorganisms capable of transforming validamycins A, D and validoxylamine A

Microorganisms: Stock cultures in our laboratories and newly isolated microorganisms were subjected to screening of strains.

Basal media: The following basal media were used in this study.

Medium A: peptone 0.2%, K_2HPO_4 0.1%, NaCl 0.5% (pH 7.0)

Medium B: yeast extracts 0.05%, KH_2PO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, $(NH_4)_2SO_4$ 0.3% (pH 5.7)

Medium C: yeast extracts 0.05%, NH_4NO_3 0.5%, KH_2PO_4 0.5%, $MgSO_4 \cdot 7H_2O$ 0.2% (pH 7.0)

(1) Media for microbial hydrolysis of validamycins

Medium for bacteria: Medium A plus validamycin A 0.1% and validamycin D 0.1%

Medium for yeast: Medium B plus validamycin A 0.1% and validamycin D 0.1%

Medium for mold: Medium C plus validamycin A 0.1% and validamycin D 0.1%

(2) Media for microbial glycosidation of validoxylamine A

Medium for bacteria: Medium A plus validoxylamine A 0.1%

Medium for yeast: Medium B plus validoxylamine A 0.1%

In each medium, one of the following disaccharides; maltose 1%, cellobiose 1% and lactose 1% is added.

Cultivation and assay

A test strain was inoculated in the medium described above (each 5 ml in test-tube for the first screening test and each 30 ml in Erlenmeyer flasks for the preparation of transformation products) and incubated for 4~8 days at 28°C (for bacteria and yeast) or 24°C (for mold) under shaking conditions. The filtrate (0.1 ml) was evaporated to dryness *in vacuo*. The residue was dissolved in pyridine (0.1 ml) and silylated with bis(trimethylsilyl)acetamide and trimethylchlorosilane. Validamycins A and D were qualitatively analyzed by gas liquid chromatographic method.[8]

Isolation of microbial transformation products

The filtrates were adsorbed on a column of activated carbon and eluted with water containing 7% *n*-butanol. The eluate was adsorbed on a column of Dowex 50 W×2(H^+ form), and the column was eluted with 0.5 N ammonia water. After concentration to dryness, the residue was chromatographed on a column of Dowex 1×2(OH^- form) by the use of water as developing solvent. The differential refractometer was used for the monitoring of the elution curve and each fraction was checked by the gas chromatographic assay.[8] The combined fractions containing desired compound were concentrated *in vacuo* to dryness.

Preparation of ^{14}C-labeled validamycin A

An improved strain of *Streptomyces hygroscopicus* var. *limoneus* was used through these studies. A loopful amount of the stock culture was inoculated into a 200-ml Erlenmeyer flask containing 30 ml of the seed medium described in the previous paper,[2] and incubated on a rotary shaker at 32°C for 48 hours. One milliliter of the seed culture was introduced into 200-ml Erlenmeyer flask containing 25~30 ml of the production medium.[2] The production flask was incubated at 32°C on a rotary shaker for 7 days. The ^{14}C-labeled compound (D-[U-^{14}C]glucose, [^{14}C]validoxylamine A) was added aseptically to the culture of various age.

The culture broth obtained from the three 200-ml Erlenmeyer flasks was centrifuged. The mycelia were washed with water. The supernatant and washings were passed through columns of Amberlite IR-120 (H^+ form, 10 ml) and Amberlite IR-45 (OH^- form, 10 ml) successively. The effluent was subjected to a chromatography on Dowex 50 W×2(H^+ form, 5 ml). [^{14}C] Validamycin A was eluted with 0.5 N ammonia water, and the crude [^{14}C]validamycin A was purified by Dowex 1×2(OH^- form, 80 ml) chromatography using water as developing solvent.

Degradation of [^{14}C]validamycin A by *Pseudomonas denitrificans*

A cell suspension of *Pseudomonas denitrificans* was inoculated in 20 ml of the medium (composed of glucose 2%, yeast extract 0.1%, peptone 1%, K_2HPO_4 0.5%, KH_2PO_4 0.1%, NaCl 0.2%, $MgSO_4 \cdot 7H_2O$ 0.02%, pH 7.2) in a 100-ml Erlenmeyer flask and cultured on a rotary shaker at 28°C for 24 hours. This broth was transferred to a 1-liter Erlenmeyer flask containing 300 ml of the medium and incubated on a rotary shaker at 28°C for 72 hours. The cells were harvested by centrifugation and washed several time with water.

[U-^{14}C]Validamycin A (2.03 g, 73.1 μCi) was dissolved in water (2 liters) and the cells harvested from the culture solution (4 liters) of *Pseudomonas denitrificans* were suspended in

the reaction solution (pH 7.1). The incubation was carried out at 28°C for 8 hours under shaking condition.

The supernatant solution (42.00μCi) was passed through a column of Amberlite IRC-50 (H^+ form, 300 ml) to absorb the basic degradation products, the column was eluted with 0.5 N ammonia water and the eluate (23.24μCi) was concentrated to dryness. The residue was separated into three components; validamine (2.008μCi), valienamine (5.873μCi) and validoxylamine A (8.070μCi) by Dowex 1×2 (OH^- form) ion-exchange resin chromatography using water as developing solvent.

Specific activities of [^{14}C]validamine and [^{14}C]valienamine were measured in the forms of crystalline validamine monohydrochloride[8] and pentaacetylvalienamine,[9] respectively.

Acid hydrolysis of [^{14}C]validamycin A

[^{14}C]Validamycin A (about 100 mg) in water (10 ml) was refluxed with Dowex 50 W×8 (H^+ form, 1 ml) for 6 hours. The reaction mixture was poured on the column of Dowex 50 W×8 (H^+ form, 1 ml) and washed with water. The effluent and washings containing D-[^{14}C]glucose were concentrated and purified by Dowex 1×2 (OH^- form, 0.5 ml) column chromatography to give D-[^{14}C]glucose.

The Dowex 50 W×8 column was eluted with 0.5 N ammonia water. The eluate containing [^{14}C]validoxylamine A was evaporated to dryness and recrystallized with water-ethanol to give crystalline [^{14}C]validoxylamine A.

Measurement of radioactivity

Radioactivities were measured by Aloka LSC-502 liquid scintillation spectrometer in dioxane-naphthalene solution (naphthalene 1 g, PPO 120 mg, POPOP 3 mg, dioxane 7.2 ml, toluene 1.35 ml, methanol 0.45 ml). Aloka thin-layer chromatogram scanner Model TRM-1B was used for radio scanning of the chromatoplate.

Results and Discussion

Microbial Hydrolysis of Validamycins A and D to Validoxylamine A

Screening for microorganisms decomposing validamycins was carried out as described above and the microorganisms were classified into three groups.

Group A: The microorganisms capable of hydrolyzing validamycin A into validoxylamine A; *Erwinia aroideae*, *Corynebacterium aquaticum*, *Oospora destructor*, *Rhizopus stolonifer*, *Syncephalastrum* sp.

Group B: The microorganisms capable of hydrolyzing validamycin D into validoxylamine A; *Endomycopsis fibuliger*, *E. chodatii*, *Endomyces decipiens*, *Candida intermedia*, *Candida* sp.

Group C: The microorganisms capable of hydrolyzing both validamycins A and D into validoxylamine A which is further decomposed; *Pseudomonas denitrificans*, *Ps. maltophilia*, *Ps. melanogenum*.

The interrelation between the degradation of validamycin A or D and the formation of validoxylamine A by the microbial transformation reaction was summarized in Table 3.

As shown in Table 3, the formation of validoxylamine A was increased in proportion to the degradation of validamycin A or D in course of time, and the produced validoxylamine A was not practically decomposed by the strains that belong to the group A or B. However, validamycins A and D were decomposed rapidly by the strains that belong to group C, and the presence of validoxylamine A was observed only in the early phase of cultivation, because the resulting validoxylamine A was further decomposed thoroughly *via* validamine and valienamine.

Microbial Hydrolysis of Validamycins C, E and F to Validamycin A

The transformation of validamycin C to validamycin A was carried out with the strains

Table 3. Degradation of validamycins A, D and formation of validoxylamine A by various strains of microorganisms

Microorganisms were cultivated on a shaker with 30 ml of each basal medium containing validamycins A and D, 0.1 %.

Organism	Validamycin A (validoxylamine A)* (mcg/ml)			Validamycin D (validoxylamine A)* (mcg/ml)		
	2 Days	4 Days	6 Days	2 Days	4 Days	6 Days
Erwinia aroideae IFO 3830	580 (220)	320 (430)	+ (720)	940 (+)	960 (+)	980 (+)
Corynebacterium aquaticum IFO 12154	830 (60)	640 (95)	550 (160)	990 (+)	980 (+)	980 (+)
Oospora destructor IFO 8556	640 (100)	580 (100)	440 (150)	1,000 (+)	980 (50)	1,000 (50)
Rhizopus stolonifer	800 (50)	480 (250)	+ (640)	1,000 (+)	960 (50)	960 (+)
Syncephalastrum sp.	710 (100)	640 (160)	360 (270)	950 (+)	920 (50)	920 (50)
Candida intermedia	1,000 (0)	1,000 (0)	1,000 (0)	720 (180)	420 (380)	350 (500)
Candida sp.	1,000 (0)	1,000 (0)	1,000 (0)	720 (180)	400 (380)	350 (520)
Endomycopsis fibuligera IFO 0109	1,000 (0)	1,000 (0)	1,000 (0)	900 (50)	680 (180)	470 (320)
〃 *chodatii* IFO 6130	940 (+)	820 (50)	880 (100)	820 (100)	260 (420)	280 (430)
Endomyces decipiens IFO 0102	1,000 (0)	1,000 (0)	1,000 (0)	900 (50)	850 (160)	840 (160)
Pseudomonas denitrificans	0 (+)	0 (+)	0 (0)	0 (+)	0 (0)	0 (0)
〃 *maltophilia* IFO 12690	0 (50)	0 (+)	0 (0)	0 (+)	0 (0)	0 (0)
〃 *melanogenum* IFO 12020	+ (50)	0 (+)	0 (0)	0 (+)	0 (0)	0 (0)

* The figures in parentheses show the quantities of validoxylamine A which were produced by the hydrolyses of validamycin A or D.

that belong to group B. As is expected, the α-glucosidic linkage in validamycin C which has both α- and β-glucosidic linkages, was selectively hydrolyzed to give validamycin A. This transformation has important significance because validamycin C is about 1,000 times less active than validamycin A against *Pellicularia sasakii* in the " dendroid-test ".[3]

Validamycins E and F were also hydrolyzed to give validamycin A by using the strains that belong to group B. Typical runs were summarized in Table 4.

Incorporation of D-[U-^{14}C]glucose into Validamycin A

In a typical run, D-[U-^{14}C]glucose (70.66μCi) was added into 30 ml of the culture broth after 1 day from inoculation, and the fermentation and purification was carried out as described above to give [^{14}C]validamycin A (specific activity 35.05μCi/g, incorporation rate 10.27 %). And also, it was shown that incorporation rate and specific activity appreciably varied by small differences of fermentation conditions.

Effect of the Culturing Time of D-[U-^{14}C]glucose Addition on the Distribution of Radioactivity in Validamycin A

When D-[U-^{14}C]glucose was added after 1 day from inoculation, validoxylamine A moiety

Table 4. Conversion of validamycins C, E, F to validamycin A by various strains of group B Microorganisms were cultivated for 6 days at 28°C on a shaker with 30 ml of each basal medium containing Validamycin C, E or F, 0.1%

Organism	Yield of validamycin A		
	VM-C	VM-E	VM-F
Endomycopsis fibuliger IFO 0109	63%	76	70
〃 *chodatii* IFO 6130	84	89	42
Endomyces decipiens IFO 0102	78	86	82
Candida intermedia	93	85	76
〃 sp.	87	85	80

was little more labeled than the D-glucose moiety. But the result from feeding of D-[U-^{14}C] glucose after 5 days from inoculation showed that the D-glucose moiety was more labeled than the validoxylamine A moiety as shown in Table 2.

Incorporation of [^{14}C]Validoxylamine A into Validamycin A

The method used here was virtually identical with that for the incorporation of D-[U-^{14}C]glucose. After incubation for 1 day, [^{14}C]validoxylamine A 124.1 mg (39.44 μCi/g) was added to 50 ml of culture broth which was further incubated for 6 days. After purification, [^{14}C]validamycin A 209.2 mg (specific activity 4.077 μCi/g, incorporation rate 14.25%) was obtained.

The [^{14}C]validamycin A 330 mg (0.55 μCi/g*) was hydrolyzed as in the foregoing experiment. Practically all radioactivity was found in the validoxylamine A moiety (0.1758 μCi, 96.86%) and almost no incorporation into D-glucose moiety (0.0015 μCi) was noted. These results suggest that validoxylamine A is produced prior to formation of β-D-glucosidic linkage and is utilized as a direct precursor by the validamycin A-producing *Streptomyces*.

Synthesis of Validamycin D by Microbial Transglucosidation

As the results described above gave the possibility of the synthesis of validamycins from validoxylamine A by microbial transglucosidation, the screening for the microorganisms possessing the ability to convert validoxylamine A to validamycin D in the presence of maltose as a glucosyl donor was carried out and the strains shown in Table 5 were isolated. The

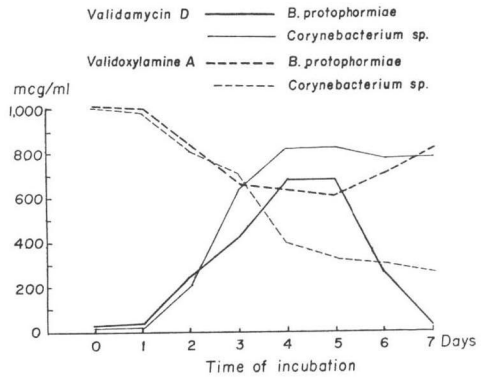
Table 5. Production of validamycin D in the maltose medium by various strains of bacteria

Organism	Growth	Validamycin D (mcg/ml)		
		2 days	4 days	6 days
Brevibacterium protophormiae IFO 12128	++	130	450	370
〃 *saperdae* 12129	++	180	+	+
〃 *divaricatum*	+	50	+	+
Corynebacterium nephridii ATCC 11425	++	190	600	400
〃 sp.	+	+	+	750
Micrococcus sp. No. 101	++	90	0	0
〃 No. 102	++	180	250	300

* This compound was obtained by the dilution of the [^{14}C]-validamycin A which was obtained by the incorporation of [^{14}C]-validoxylamine A.

relationship between the decrease of validoxylamine A and the increase of validamycin D was shown in Fig. 1. In the case of *Brevibacterium* sp., the maximum yield of validamycin D was obtained after approximately 3 to 5 days incubation and the yield of validamycin D was decreased by further cultivation. While in the case of *Corynebacterium* sp., the yield of validamycin D was little effected by prolonged cultivation.

Fig. 1. Production of validamycin D in the maltose medium by *Brevibacterium protophormiae* and *Corynebacterium* sp.



Synthesis of Validamycin A by Microbial Transglucosidation Reaction

Screening test of β-D-glucosidation of validoxylamine A was carried out in a medium containing cellobiose as a β-glucosyl donor. The strains found in the present studies were *Rhodotorula glutinis*, *R. marina*, *R. lactosa* and *R. rubra* as shown in Table 6.

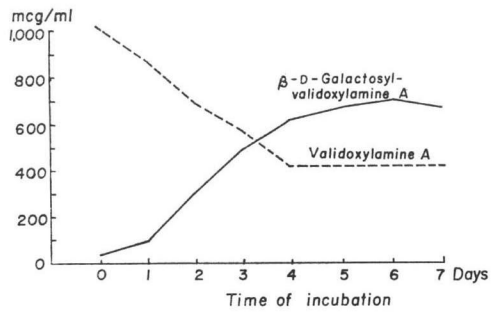
Table 6. Production of validamycin A in the cellobiose medium by various strains of yeast.

Organism	Growth	Validamycin A (mcg/ml, 4 days)
Rhodotorula glutinis IFO 1382	##	160
〃 *marina* 1421	#	+
〃 *lactosa* 1424	#	50
〃 *rubra* 1502	#	+

Synthesis of β-D-Galactosyl-validoxylamine A by Microbial Transglycosidation

Success in microbial semi-synthesis of validamycins A and D gave a possibility for the synthesis of new validamycins, and the synthesis of β-D-galactosyl-validoxylamine A, a new validamycin from validoxylamine A was carried out in a medium containing lactose as a β-galactosyl donor using the strains that are capable of synthesizing validamycin A from validoxylamine A in a medium containing cellobiose as a β-glucosyl donor.

Fig. 2. Production of β-D-galactosyl-validoxylamine A in the lactose medium by *Rhodotorula glutinis*



Rhodotorula glutinis was incubated at 28°C for 6 days in the medium (2.5 liters) containing validoxylamine A (0.1 %) as an acceptor and lactose (0.1 %) as a β-galactosyl donor. Time course in β-D-galactosyl-validoxylamine A production was in shown in Fig. 2. β-D-Galactosyl-validoxylamine A (1.16 g) was obtained as white amorphous powder. Found: C, 46.32; H, 7.30; N, 2.84 %. Calcd. for $C_{20}H_{35}NO_{13}\cdot H_2O$: C, 46.60; H, 7.24; N, 2.72, $[\alpha]_D^{23}+117.4°$ (*c* 1, H_2O), NMR (D_2O); δ 4.56 (d, J=8 Hz, anomeric proton).

Characterization of β-D-Galactosyl-validoxylamine A

Hydrolysis of β-D-galactosyl-validoxylamine A with 2 N H_2SO_4 (80°C, 8 hours) regenerated validoxylamine A and D-galactose, and acetylation (acetic anhydride in pyridine) of β-D-galactosyl-validoxylamine A gave octaacetyl-β-D-galactosyl-validoxylamine A. Found: C, 52.13; H, 5.92; N, 1.43%. Calcd. for $C_{42}H_{57}NO_{24}$: C, 52.55; H, 6.16; N, 1.60. Mass spectrum *m/e*: 959 (M^+) and 960 (M^++1).

As described above, the selective hydrolysis of the glucosidic linkages (α- and β-types) of validamycins and biological semi-syntheses of validamycins was achieved by several microorganisms by using validoxylamine A as an acceptor and a disaccharide as a glycosyl donor.

β-D-Galactosyl-validoxylamine A, a new validamycin showed unfortunately less activity than validamycin A, however, the possibility of biological semi-synthesis of new validamycins was offered.

Although no definite information is available as to how the glucosidic bonds are formed in the biosynthesis of validamycin A and whether validoxylamine A is an intermediate or a shunt product of validamycin A biosynthesis in the mycelia of *S. hygroscopicus* var. *limoneus*, it is most probable that validoxylamine A or its activated derivatives was biosynthesized at first and then followed by a transglucosidation reaction to produce validamycins A, C, D, E and F.

Acknowledgements

We are grateful to Drs. R. TAKEDA, A. MIYAKE and M. ISONO for their encouragement and advice. We also wish to thank Drs. I. BANNO, E. HIGASHIDE and T. IWASA for the important advice and Messrs. K. KAWAHARA and T. MATSUYAMA for their technical assistance.

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) IWASA, T; E. HIGASHIDE, H. YAMAMOTO & M. SHIBATA: Studies on validamycins, new antibiotics. II. Production and biological properties of validamycins A and B. J. Antibiotics 24: 107~113, 1971
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) IWASA, T.; Y. KAMEDA, M. ASAI, S. HORII & K. MIZUNO: Studies on validamycins, new antibiotics. IV. Isolation and characterization of validamycins A and B. J. Antibiotics 24: 119~123, 1971
5) HORII, S.; T. IWASA & Y. KAMEDA: Studies on validamycins, new antibiotics. V. Degradation studies. J. Antibiotics 24: 57~58, 1971
6) HORII, S.; T. IWASA, E. MIZUTA & Y. KAMEDA: Studies on validamycins, new antibiotics. VI. Validamine, hydroxyvalidamine and validatol, new cyclitols. J. Antibiotics 24: 59~63, 1971
7) KAMIYA, K.; Y. WADA, S. HORII & M. NISHIKAWA: Studies on validamycins, new antibiotics. VII. The X-ray analysis of validamine hydrobromide. J. Antibiotics 24: 317~318, 1971
8) HORII, S.; Y. KAMEDA & K. KAWAHARA: Studies on validamycins. VIII. Isolation and characterization of validamycins C, D, E and F. J. Antibiotics 25: 48~53, 1972
9) KAMEDA, Y. & S. HORII: The unsaturated cyclitol part of the new antibiotics, the validamycins. J. C. S. Chem. Comm. 1972: 746~747, 1972
10) HORII, S. & Y. KAMEDA: Structure of the antibiotic validamycin A. J. C. S. Chem. Comm. 1972: 747~748, 1972
11) NISHI, K.; A. TAKABATAKE, M. HATTORI, O. WAKAE & K. MATSUURA: Determination of residues

of validamycin A in soil. Ann. Phytopath. Soc. Japan 39: 169, 1973
12) SEBEK, O. K. & D. PERLMAN: Microbial transformations of antibiotics. Advan. Appl. Microbiol. 14: 123~150, 1971
13) SEBEK, O. K.: Microbial conversion of antibiotics. Lloydia 37: 115~133, 1974
14) DEMAIN, A. L. & E. INAMINE: Biochemistry and regulation of streptomycin and mannosidostreptomycinase (α-D-mannosidase) formation. Bacteriol. Rev. 34: 1~19, 1970
15) ENDO, T. & D. PERLMAN: Transglycosylation of neamine. J. Antibiotics 25: 681~682, 1972
16) ENDO, T. & D. PERLMAN: The transglycosidation of kanamycin A. J. Antibiotics 25: 751, 1972