CONTROLLED BIOSYNTHESIS OF NEOVIRIDOGRISEINS, NEW HOMOLOGUES OF VIRIDOGRISEIN

I. TAXONOMY AND FERMENTATION*

YASUSHI OKUMURA, KAZUHIKO OKAMURA, TOMOKO TAKEI, KAGEAKI KOUNO, JOSEPH LEIN*, TOMOYUKI ISHIKURA and YASUO FUKAGAWA

Central Research Laboratories, Sanraku-Ocean Co., Ltd.
9-1 Johnan 4 Chome, Fujisawa-shi 251, Japan
*Panlabs, Inc., Fayetteville, New York 13066, U.S.A.

(Received for publication December 26, 1978)

Neoviridogriseins, new homologues of viridogrisein, were produced with viridogrisein and griseoviridin by *Streptomyces* sp. P8648 which was identified as a strain of *Streptomyces griseoviridus*.

In the course of screening for new antibiotics, we isolated a strain of streptomycete named *Streptomyces* sp. P8648 which produced antibiotics active against some Gram-positive bacteria and mycoplasmas. They were recovered from broth filtrate and identified as viridogrisein[1] (=etamycin) and griseoviridin[2] on the basis of their physico-chemical and antimicrobial properties. In the meantime, *Streptomyces* sp. P8648 was taxonomically concluded to be a strain of *Streptomyces griseoviridus*[3]**, though some minor differences were observed between the type culture of *Streptomyces griseoviridus* (NRRL2427) and our isolate, as is described in this paper. *Streptomyces* sp. P8648 produced viridogrisein, griseoviridin and three minor factors named neoviridogriseins I, II and III under normal fermentation conditions. The yield improvement of the minor factors was tried by the so-called directed or controlled biosynthesis with amino acids.

According to the classification proposed by TANAKA[4] and by VAZQUEZ[5], the mikamycin or streptogramin family of antibiotics is divided into group A and group B which are antimicrobially synergistic. Group A is a non-peptidyl, macrocyclic lactone antibiotic and includes mikamycin A[6] (=ostreogrycin A[7]=virginiamycin M[8]=pristinamycin II_A[9]=streptogramin A[5]=vernamycin A[10]=synergistin A-1[11]), ostreogrycin G[12] (=virginiamycin M_2[13]=pristinamycin II_B[9]=dihydroostreogrycin A[12]), compounds A-2315A, A-2315B and A-2315C[14], madumycins I and II[15,16] and griseoviridin[2]. The structures of these antibiotics are shown in Fig. 1.

Group B includes depsipeptide antibiotics which are further classified into sub-group B I and sub-group B II, based on the number of constituent moieties. As antibiotics in sub-group B I which are composed of seven moieties, that is, 3-hydroxypicolinic acid, L-threonine, L-proline, L-phenylglycine and three amino acids, there are many members known like mikamycin B[6] (=vernamycin B_α[17]=streptogramin B[5]=pristinamycin I_A[9]=ostreogrycin B[18]), vernamycin B_β[17] (=ostreogrycin B_2[8]=pristinamycin I_B[9]), vernamycin B_γ[17] (=ostreogrycin B_1[18]=pristinamycin I_C[9]), vernamycin B_δ[17],

* This and next papers were presented at the 208th Scientific Meeting of Japan Antibiotics Research Association, Jan. 30, 1978 (Tokyo).

** *Streptomyces griseoviridis* ANDERSON, EHRLICH, SUN and BURKHOLDER (8th Ed., BERGEY'S Manual of Determinative Bacteriology, The Williams & Wilkins Company, Baltimore, 1974).

ostreogrycin B_3[19], patricins A and B[20], vernamycin C[21] (=doricin[21]), virginiamycin S[22], and virginiamycins S_2, S_3 and S_4[23]. The structures of sub-group B I antibiotics are summarized in Fig. 2.

In contrast, viridogrisein[2] (=etamycin[24]) has been the sole member in sub-group B II, and contains eight constituent moieties, that is, 3-hydroxypicolinic acid, L-threonine, D-leucine, *allo*-hydroxy-D-proline, sarcosine, N,β-dimethyl-L-leucine, L-alanine and L-phenylsarcosine (Fig. 3). According to the literature, the antibiotics in subgroup B I are most often produced in mixtures of more than two components, while viridogrisein has been reported as a single component.

So-called directed or controlled biosynthesis of antibiotics has been extensively studied for actinomycin[25], gramicidin S[26] and tyrocidine[27], and many homologues of these antibiotics were biochemically produced. Enzyme systems responsible for the biosynthesis of these antibiotics seem to have a loose substrate specificity which results in the production of a diversity of homologues.

This series of studies deal with the controlled biosynthesis of neoviridogriseins I, II and III, viridogrisein (neoviridogrisein IV) and griseoviridin (Fig. 1A). The structures of these components are illustrated in Fig. 4.

Fig. 1. Structures of mikamycin A group antibiotics.

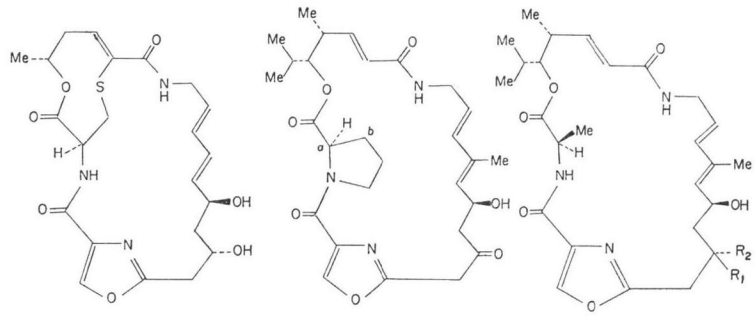
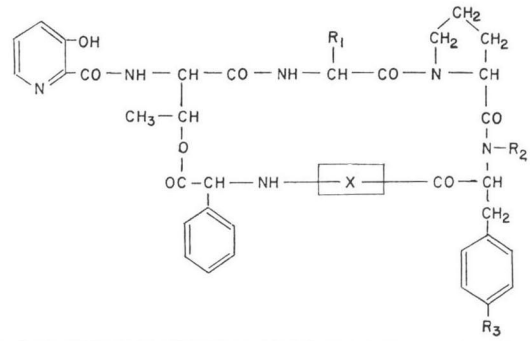


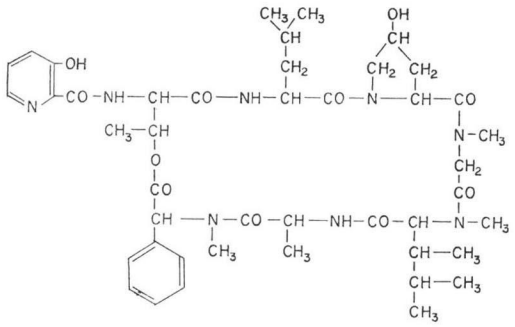
Fig. 2. Structures of mikamycin B group (sub-group B I) antibiotics.



Antibiotic	R_1	R_2	R_3	X
Mikamycin B	$-C_2H_5$	$-CH_3$	$-N(CH_3)_2$	4-oxopipecolic acid
Vernamycin B_β	$-C_2H_5$	$-CH_3$	$-NHCH_3$	4-oxopipecolic acid
Vernamycin B_γ	$-CH_3$	$-CH_3$	$-N(CH_3)_2$	4-oxopipecolic acid
Vernamycin B_δ	$-CH_3$	$-CH_3$	$-NHCH_3$	4-oxopipecolic acid
Ostreogrycin B_3	$-C_2H_5$	$-CH_3$	$-N(CH_3)_2$	3-hydroxy-4-oxopipecolic acid
Patricin A	$-C_2H_5$	$-CH_3$	$-H$	proline
Patricin B	$-C_2H_5$	$-CH_3$	$-H$	pipecolic acid
Vernamycin C	$-C_2H_5$	$-CH_3$	$-N(CH_3)_2$	aspartic acid
Virginiamycin S	$-C_2H_5$	$-CH_3$	$-H$	4-oxopipecolic acid
Virginiamycin S_2	$-C_2H_5$	$-H$	$-H$	4-hydroxypipecolic acid
Virginiamycin S_3	$-C_2H_5$	$-CH_3$	$-H$	3-hydroxy-4-oxopipecolic acid
Virginiamycin S_4	$-CH_3$	$-CH_3$	$-H$	4-oxopipecolic acid

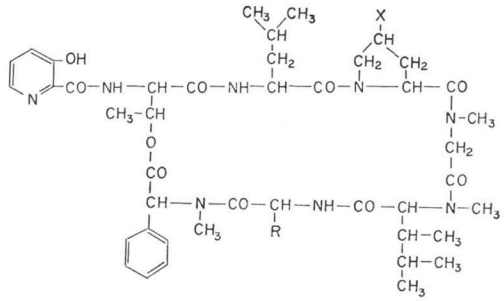
In particular, the present paper deals with the taxonomy of *Streptomyces* sp. P8648, as well as the fermentation and identification of viridogrisein and griseoviridin.

Fig. 3. Structure of mikamycin B group (sub-group B II) antibiotic.



Viridogrisein (= Etamycin)

Fig. 4. Structures of neoviridogriseins I, II, III, and IV.



	R	X
Neoviridogrisein I	$-CH_2CH_3$	-H
Neoviridogrisein II	$-CH_3$	-H
Neoviridogrisein III	$-CH_2CH_3$	-OH
Neoviridogrisein IV (= Viridogrisein)	$-CH_3$	-OH

Materials and Methods

1. Materials

(1) Antibiotics and reagents

Viridogrisein and griseoviridin were kindly supplied by Parke, Davis & Co., Detroit 32, Mich., U.S.A. and etamycin by Bristol Laboratories, Inc., Syracuse, New York, U.S.A. Pre-coated silica gel plates (Pre-Coated TLC Plates Silica Gel 60 F-254) and silica gel for column chromatography (Kieselgel 60 Art. 7734) were obtained from E. Merck, Darmstadt, Germany; pre-coated cellulose sheets (Eastman Chromagram Sheet No. 6065) from Eastman Kodak Co., Rochester, New York 14650, U.S.A.; and Sephadex LH-20 from Pharmacia Fine Chemicals AB, Uppsala, Sweden. Other materials were purchased from commercial sources.

(2) Microorganisms

Streptomyces griseus (NRRL2426) was obtained from Northern Regional Research Laboratories, Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois, U.S.A.

S. griseoviridus (NRRL2427), *S. griseoroseus* and *S. ostreogriseus* were kindly supplied by Dr. A. SEINO, Kaken Chemical Co., Tokyo, Japan.

2. Methods

(1) Taxonomical methods

The morphological and physiological characteristics of the streptomycete were determined according to the ISP (International Streptomyces Project) procedure[28].

(2) Disc-assay method

The potency of broth was determined by the paper-disc method using *Staphylococcus aureus* FDA 209P and *Sarcina lutea* as test organisms. Assay plates were prepared as follows:

One loopful of the test organism on a nutrient agar slant was inoculated into a 100-ml Erlenmeyer flask containing 20 ml of 2% nutrient broth and incubated at 28°C for 16 hours on a rotary shaker. This culture was diluted 66 times in 2% nutrient broth and 0.5 ml of the dilution was added to 50 ml of molten nutrient agar. It was poured on top of a 100-ml layer of the basal agar medium (1% nutrient broth and 2% agar) in a rectangular assay dish (16 cm × 24 cm). Paper discs (Toyo paper disc, 8 mm in diameter, thin, Toyo Roshi Co., Toyo, Japan) were soaked in broth filtrate or antibiotic solutions and, after removal of excessive volume on filter paper, applied on the assay plate. After overnight incubation at 37°C, the inhibition zones were read in mm.

(3) Thin-layer chromatography

Two milliliters of broth filtrate were extracted with 2 ml of ethylacetate. The extract was evaporated to dryness and dissolved in 0.1 ml of methanol. A suitable amount of the solution was spotted on a silica gel TLC plate and developed with $CHCl_3$ - MeOH (30: 1). The antibiotics were detected by irradiation under UV-light (365 nm) and/or by bioautography using *Staphylococcus aureus* FDA 209P or *Sarcina lutea*. Typical thin-layer chromatogram and bioautogram are shown in Fig. 5.

(4) Fermentation

Seed culture medium (medium LL-3) consisting of 0.5% soy bean meal, 0.5% peanut meal, 0.5% oat meal, 0.5% dry yeast and 0.5% beet molasses was adjusted to pH 6.5 and distributed in 50-ml amounts in 250-ml Erlenmeyer flasks. After sterilization in an autoclave at 120°C for 15 minutes, each flask was inoculated with a loopful of *Streptomyces* sp. P8648 growth on a glucose-yeast extract-malt extract agar slant and the flasks were incubated at 28°C on a rotary shaker at 220 rpm. Broth potency was determined by the disc-assay method. Cell growth was measured as packed volume of mycelium per ml broth. Typical time course of fermentation is shown in Fig. 6.

(5) Isolation

Ten liters of the harvested broth (medium LL-3′: 0.5% soy bean meal, 0.5% cotton seed meal, 0.5% oat meal, 0.5% dry yeast and 0.5% beet molasses) were filtered and the filtrate was extracted at pH 8.0 with ethylacetate (3 liters × 2). The extract was dried over sodium sulfate (anhydrous) and evaporated to dryness under reduced pressure. From the dry material, neoviridogriseins including viridogrisein (mainly) were extracted with toluene (100 ml × 2), while griseoviridin remained in the residue. The extract was evaporated to dryness and charged on a silica gel column (3 cm × 50 cm) which was developed with benzene - MeOH (20 : 1). Antimicrobial activity was monitered by thin-layer chromatography followed by bioassay. Active materials were separated into four groups. Factors 1, 2 and 3 which were present in very small quantities were named neoviridogriseins I, II and III respectively, whereas Factor 4, the main component, was named neoviridogrisein IV which is identical with viridogrisein. Relative yields were; Factor 1: Factor 2: Factor 3: Factor 4 = 1: 25: 4: 430.

The toluene extraction residue, containing Factor 5, was dissolved in a small amount of methanol and applied on a Sephadex LH-20

Fig. 5. Diagrammatic thin-layer chromatogram and bioautogram of neoviridogriseins and griseoviridin.
Plate: Pre-coated Silica Gel 60 F-254 (E. Merck)
Solvent system: $CHCl_3$ - MeOH (30: 1)
Detection:
(A) UV-light (365 nm for neoviridogriseins and 254 nm for griseoviridin)
(B) Bioautography on *Sarcina lutea*
Sample:
(1) The ethylacetate extract of broth filtrate from *Streptomyces* sp. P8648 fermented in medium LL-3′
(2) Authentic viridogrisein and griseoviridin
VG*: viridogrisein, GV**: griseoviridin

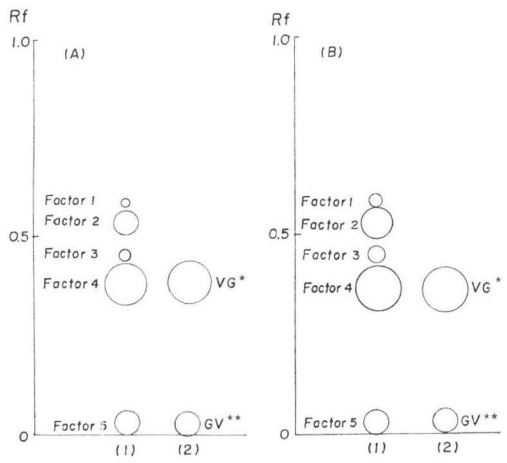
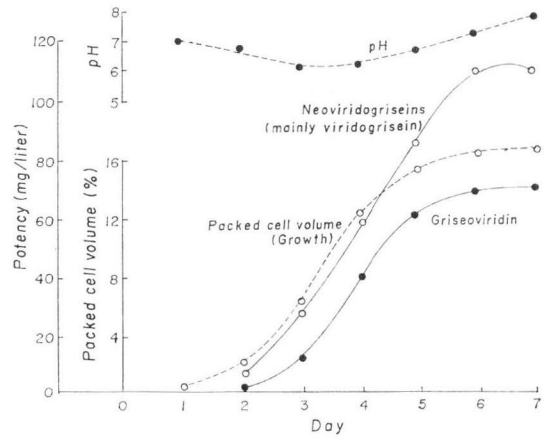


Fig. 6. Time course of fermentation of *Streptomyces* sp. P8648 in medium LL-3′ without precursor amino acids.



Scheme 1. Isolation procedure of neoviridogriseins and griseoviridin.

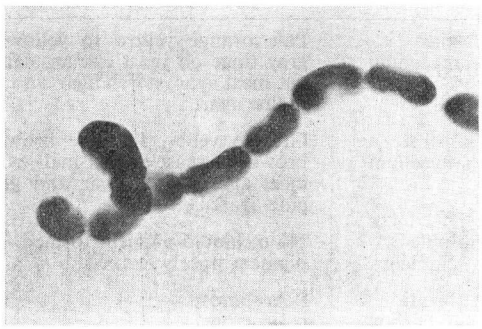
NVG*: neoviridogriseins
GV**: griseoviridin

Fermented broth (NVG*: 110 mg/liter, GV**: 70 mg/liter)
↓ filtered
Filtrate (10 liter)
↓ extracted with EtOAc at pH 8.0 (3 liters × 2)
Extract
↓ dried over Na_2SO_4 (anhydrous) and evaporated to dryness
Dry material (3.5 g)
↓ extracted with toluene (100 ml × 2)

Extract	Residue
chromatographed on silica gel	chromatographed on Sephadex LH-20
eluted with benzene - MeOH (20: 1)	eluted with MeOH
	crystallized from hot MeOH

Factor 1 (1.5mg) Factor 2 (30mg) Factor 3 (4.0mg) Factor 4 (650mg) Factor 5 (370mg)

Fig. 7. Electronmicrograph of spores of *Streptomyces* sp. P8648 (Inorganic salt-starch agar, ×25,000)



column (2 cm × 50 cm), which was then developed with methanol. Each fraction was monitered by bioassay, using *Sarcina lutea* as test organism. Active fractions were combined and evaporated to dryness. The dry material, Factor 5, was crystallized from hot methanol. The overall isolation procedure is summarized in Scheme 1.

Results

1. Microorganism

The streptomycete strain, named *Streptomyces* sp. P8648, produced colorless, short aerial mycelia from well-branched substrate mycelia. Spore chains with smooth surface (Fig. 7) formed in loose loops on top of aerial mycelia.

Streptomyces sp. P8648 was compared with various strains of *Streptomyces* which were known to produce the mikamycin group of antibiotics. Among others, the following strains were compared with our isolate: *S. griseus* (NRRL2426), *S. griseoviridus* (NRRL2427), *S. griseoroseus* (KCC-S-0232) and *S. ostreogriseus* (KCC-S-0186).

The available information on cultural and physiological characteristics indicated that *Streptomyces* sp. P8648 was different from the above-listed strains. But, among the four species, *S. griseoviridus* (NRRL2427) was the most similar to our strain. Therefore, *S. griseoviridus* (NRRL2427) and *Streptomyces* sp. P8648 were critically compared under various conditions. Table 1 shows some of the cultural and physiological differences observed between the two strains. Moreover, *Streptomyces* sp. P8648 and the authentic strain (NRRL2427) of *Streptomyces griseoviridus* were fermented in a medium containing 1% glucose, 1% soluble starch, 1% soy bean meal, 0.2% DL-α-amino-*n*-butyric acid and 0.2% L-proline. Product analysis of the broth filtrate proved that the former produced neoviridogriseins I, II and III, viridogrisein and griseoviridin, whereas the latter produced viridogrisein and griseoviridin but not neoviridogriseins I, II and III.

2. Fermentation

Several carbon and nitrogen sources were examined for increased production of neoviridogriseins. It is clear from the results shown in Tables 2 and 3 that among the test sources glucose was

Table 1. Comparison of *Streptomyces* sp. P8648 with *Streptomyces griseoviridus* NRRL2427.

	Streptomyces griseoviridus NRRL2427	*Streptomyces* sp. P8648
Aerial mycelium	Pale orange yellow to yellowish pink with gray tinge on yeast extract-malt extract agar, oat meal agar, starch agar and glycerin-asparagine agar.	White to grayish white aerial mycelium slightly formed on most ISP media.
Substrate mycelium	Grayish yellow to olive brown or blackish brown on yeast extract-malt extract agar, oat meal agar, starch agar and glycerin-asparagine agar.	Pale yellow to grayish brown on most ISP media.
Soluble pigment	No melanoid pigment formed. Rarely yellow pigment poorly formed.	No melanoid pigment formed. Rarely brown pigment slightly formed.
Utilization of carbon sources	L-Arabinose +++ D-Fructose ± Sucrose −	L-Arabinose − D-Fructose +++ Sucrose ±
Production of antibiotics	Viridogrisein and griseoviridin.	Neoviridogriseins I, II and III together with viridogrisein and griseoviridin.

Table 2. Effect of the carbon source on the production of neoviridogriseins and griseoviridin.

Carbon source (2%)	Basal medium A			Basal medium B		
	pH	NVG* (μg/ml)	GV** (μg/ml)	pH	NVG* (μg/ml)	GV** (μg/ml)
Soluble starch	9.0	60.0	62.5	8.8	trace	12.0
Glucose	8.3	75.0	80.0	8.3	42.0	49.0
Sucrose	9.3	0	0	9.5	0	0
Corn starch	8.7	60.0	57.5	9.1	trace	6.3
Glycerin	8.5	0	0	8.7	0	0
Maltose	9.4	0	0	9.5	0	0
Beet molasses	9.5	37.5	42.0	9.5	0	0

Basal medium A: cotton seed meal 1.5%, soy bean meal 1.5%, K_2HPO_4 0.05% and $CaCO_3$ 0.2% (pH 6.5)

Basal medium B: peptone 1.0%, corn steep liquor 0.5%, soy bean meal 1.5%, K_2HPO_4 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05% and $CaCO_3$ 0.2% (pH 6.5)

NVG*: neoviridogriseins, GV**: griseoviridin

the most suitable carbon source, while soy bean meal and cotton seed meal seemed the best nitrogen sources. The combination of glucose and cotton seed meal and/or soy bean meal was studied in more detail. As a result, the following medium (medium NS-3) was employed for production of neoviridogriseins and griseoviridin: 2% glucose, 0.5% soluble starch, 1% cotton seed meal, 0.5% soy bean meal, 0.05% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$ and 0.2% $CaCO_3$.

3. Identification of Viridogrisein and Griseoviridin

Identification of Factor 4 (neoviridogrisein IV) was achieved from the following physico-chemical characteristics of Factor 4:

Melting point, 151°C; optical rotation, $[\alpha]_D^{25}$ +43.7°(*c* 1 in MeOH); molecular weight (determined by mass spectrometry), 878; UV absorption, λ_{max} 304 nm (in MeOH, $E_{1cm}^{1\%}$ 90) and λ_{max} 335 nm (in MeOH - 0.1 N NaOH, $E_{1cm}^{1\%}$ 96); TLC (cf. Fig. 5); mass spectrum (Fig. 8) and IR spectrum. These data proved the identity of Factor 4 with viridogrisein. Finally, two-dimensional thin-layer chromato-

Table 3. Effect of the nitrogen source on the production of neoviridogriseins and griseoviridin.

Nitrogen source (0.5%)	Basal medium C			Basal medium D		
	pH	NVG* (μg/ml)	GV** (μg/ml)	pH	NVG* (μg/ml)	GV** (μg/ml)
Soy bean meal	8.9	46.0	52.0	8.8	37.5	41.0
Cotton seed meal	9.0	49.0	57.5	8.6	7.5	33.0
Peptone	9.2	trace	trace	9.0	trace	trace
Casein	8.8	49.0	57.5	8.6	8.5	22.0
Corn steep liquor	8.8	34.0	38.0	9.0	trace	trace
Casamino acid	8.0	60.0	49.0	8.2	41.5	42.0
$(NH_4)_2SO_4$	4.6	0	0	4.5	0	0

Basal medium C: glucose 2.0%, K_2HPO_4 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05% and $CaCO_3$ 0.2% (pH 6.5)
Basal medium D: soluble starch 2.0%, K_2HPO_4 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05% and $CaCO_3$ 0.2% (pH 6.5)
NVG*: neoviridogriseins, GV**: griseoviridin

grams of total acid-hydrolysates of Factor 4 and viridogrisein confirmed their identity, too.

Identification of Factor 5 was achieved from the following physico-chemical characteristics of Factor 5:

Melting point, 185°C; optical rotation, $[\alpha]_D^{25} -230°$ (*c* 0.5 in MeOH); UV absorption, λ_{max} 220.5 nm (in MeOH, $E_{1cm}^{1\%}$ 910); molecular weight (determined by mass spectrometry), 477; TLC (cf. Fig. 5); mass spectrum (Fig. 9) and IR spectrum. These data were practically identical with the physico-chemical properties of griseoviridin.

Fig. 8. Mass spectra of Factor 4 and viridogrisein.

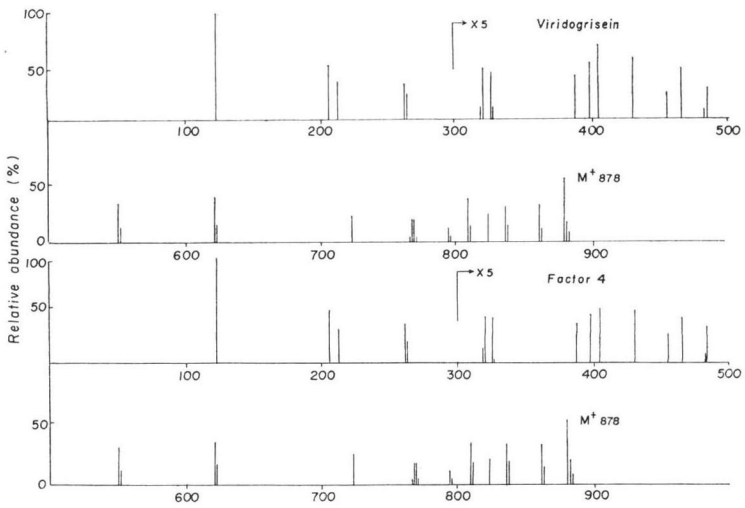
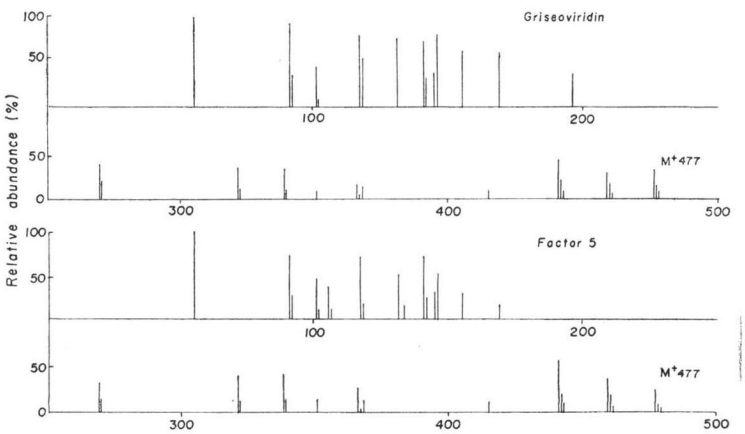


Fig. 9. Mass spectra of Factor 5 and griseoviridin.



Discussion

We isolated from a soil sample a streptomycete named *Streptomyces* sp. P8648, which produced viridogrisein[1] and griseoviridin[2]. This strain was tax-

onomically studied and identified with *Streptomyces griseoviridus*[3], though some minor differences were recorded in their taxonomical and physiological characteristics.

Generally speaking, the biosynthesis of peptide antibiotics is not directed by the ribosome-RNA system of protein synthesis. This fact is well established in the biosynthesis of actinomycin[25], gramicidin S[26], tyrocidine[27], *etc.* As described by KAMAL and KATZ[29], the production of etamycin by *Streptomyces griseoviridus* (strain 04955) was promoted by chloramphenicol, which seemed to indicate a possible participation of the thiotemplate mechanism[30] in the biosynthesis of etamycin. Because of the loose specificity of the thiotemplate system, there remain many possibilities to biochemically replace the constituent amino acid(s) in peptide antibiotics with structurally related compounds to produce novel, and hopefully more active homologues. Along this line of approach, we studied the controlled biosynthesis of neoviridogriseins by *Streptomyces* sp. P8648, and, as a result, could obtain more quantities of neoviridogriseins I, II and III. As detailed in the following papers, the two constituent amino acids (*allo*-hydroxy-D-proline and L-alanine) in viridogrisein were proved to be biochemically replaceable by D-proline and L-α-amino-*n*-butyric acid instead of *allo*-hydroxy-D-proline and L-alanine; neoviridogrisein I contains D-proline and L-α-amino-*n*-butyric acid instead of *allo*-hydroxy-D-proline and L-alanine; neoviridogrisein II D-proline instead of *allo*-hydroxy-D-proline; and neoviridogrisein III L-α-amino-*n*-butyric acid instead of L-alanine.

Acknowledgement

We express our sincere thanks to Prof. Y. YAMADA, Tokyo College of Pharmacy, Tokyo, Japan, for his help in chemical studies including mass spectrometry. We are grateful to Dr. A. SEINO, Kaken Chemical Co., Ltd., Tokyo, Japan, for gifts of the cultures; and to Dr. H. E. MACHAMER, Parke, Davis & Co., Detroit 32, Mich., U.S.A., for kindly providing viridogrisein and griseoviridin; and to Dr. I. R. HOOPER, Bristol Laboratories, Inc., Syracuse, New York, U.S.A., for an etamycin sample.

References

1) HASKELL, T. H.; A. MARETZKI & Q. R. BARTZ: Viridogrisein: Chemical studies. Antibiotics Annual 1954/1955: 784~789, 1955
2) BARTZ, Q. R.; J. STANDIFORD, J. D. MOLD, D. W. JOHANNESSEN, A. RYDER, A. MARETZKI & T. H. HASKELL: Griseoviridin and viridogrisein: Isolation and characterization. Antibiotics Annual 1954/1955: 777~783, 1955
3) BARTZ, Q. R.; J. EHRLICH, M. P. KNUDSEN & R. M. SMITH (Parke, Davis & Co.): Viridogrisein and its fermentative production with griseoviridin. U.S. Pat. 3,023,204, Feb. 27, 1962
4) TANAKA, N.: Mikamycin. *in* Antibiotics. Vol. III, pp. 487~497, Springer-Verlag, New York, 1975
5) VAZQUEZ, D.: The streptogramin family of antibiotics. Antibiotics. Vol. III, pp. 521~534, Springer-Verlag, New York, 1975
6) ARAI, M.; S. KARASAWA, H. YONEHARA & H. UMEZAWA: Studies on mikamycin. J. Antibiotics, Ser. A11: 14~20, 1958
7) DELPIERRE, G. R.; F. W. EASTWOOD, G. E. GREAM, D. G. I. KINGSTON, P. S. SARIN, L. TODD & D. H. WILLIAMS: Antibiotics of the ostreogrycin complex. II. Structure of ostreogrycin A. J. Chem. Soc. (C) 1966: 1653~1669, 1966
8) VANDERHAEGHE, H.; P. V. DIJCK, G. PARMENTIER & P. D. SOMMER: Isolation and properties of the components of staphylomycin. Antibiot. & Chemoth. 7: 606~614, 1957
9) JOLLES, G.; B. TERLAIN & T. P. THOMAS: Metabolic investigations on pristinamycin. Nature 207: 199, 1965
10) DENOVICK, R.; J. D. DUTCHER, L. J. HEUSER & J. F. PAGANO (Olin Mathieson Chemical Corp.): Antibiotics. U.S. Pat. 2,990,325, June 27, 1961
11) CELMER, W. D. & B. A. SOBIN: The isolation of two synergistic antibiotics from a single fermentation source. Antibiotics Annual 1955/1956: 437~441, 1956
12) KINGSTON, D. G.; P. S. SARIN, L. TODD & D. H. WILLIAMS: Antibiotics of ostreogrycin complex. IV. The structure of ostreogrycin G. J. Chem. Soc. 1966: 1856~1860, 1966
13) DESOMER, P. & P. V. DIJCK: A preliminary report on antibiotic number 899, streptogramin-like substance. Antibiot. & Chemoth. 5: 632~639, 1955
14) HAMILL, R. L. & W. M. STARK (Eli Lilly and Co.): Antibiotic A-2315 and process for preparation thereof.

U.S. Pat. 3,923,980, Dec. 2, 1975
15) BRAZHNIKOVA, M. G.; M. P. KUDINOVA, N. P. POTAPOVA, T. M. FILIPPOVA, E. BOROWSKI, J. ZIELINSKI & J. GOLIC: Structure of the antibiotic madumycin. Bioorg. Khim. 2: 149, 1976
16) BYCROFT, B. W.: Configurational and conformational studies on the group A peptide antibiotics of the mikamycin (streptogramin, virginiamycin) family. J. Chem. Soc., Perkin I 1977: 2464~2470, 1977
17) BODANSZKY, M. & M. A. ONDETTI: Structure of vernamycin B group of antibiotics. Antimicr. Agents & Chemoth. -1963: 360~365, 1964
18) EASTWOOD, F. W.; B. K. SNELL & S. A. TODD: Antibiotics of the E-129 (ostreogrycin) complex. I. The structure of E-129 B. J. Chem. Soc., Chem. Comm. 1960: 2286, 1960
19) COX, B. R. & F. W. EASTWOOD: The structure of the antibiotic ostreogrycin B_3. J. Chem. Soc., Chem. Comm. 1970: 1623~1624, 1970
20) BODANSZKY, M.; M. A. ONDETTI & J. T. SHEEHAN (Squibb, E. R. & Sons, Inc.): Patricin A and B and related compounds. U.S. Pat. 3,373,151, Mar. 12, 1968
21) BODANSZKY, M. & J. T. SHEEHAN: Structure of doricin, a peptide related to vernamycin B group. Antimicr. Agents & Chemoth. -1963: 38~40, 1964
22) VANDERHAEGHE, H. & G. PARMENTIER: The structure of factor S of staphylomycin. J. Am. Chem. Soc. 82: 4414~4422, 1960
23) VANDERHAEGHE, H.; G. JANSSEN & F. COMPERNOLLE: The structure of minor components of virginiamycin S. Tetrahed. Lett. 1971: 2687, 1971
24) SHEEHAN, J. C.; H. G. ZACHAN & W. B. LAWSAN: The structure of etamycin. J. Am. Chem. Soc. 80: 3349~3359, 1958
25) KATZ, E.: Actinomycins. Antibiotics. Vol. II, pp. 276~341, Springer-Verlag, Berlin, 1967
26) SAITO, Y.; S. OTANI & S. OTANI: Biosynthesis of gramicidin S. Adv. Enzymol. Vol. 33, pp. 337~380, Interscience Publishers, New York, 1970
27) LIPMANN, F.; W. GEVERS, H. KLEINKAUF & R. ROSKOSKI: Polypeptide synthesis on protein templates: The enzymatic synthesis of gramicidin S and tyrocidine. Adv. Enzymol. Vol. 35, pp. 1~34, Interscience Publishers, New York, 1971
28) SHIRLING, E. B. & D. GOTTLIEB: Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313~340, 1956
29) KAMAL, F. & E. KATZ: Studies of etamycin biosynthesis employing short-term experiments with radiolabelled precursors. J. Antibiotics 24: 944~949, 1976
30) LIPMANN, F.: Attempts to map a process evolution of peptide biosynthesis. Science 173: 875~884, 1971