Kerriamycins A, B and C, New Isotetracenone Antibiotics

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Three new antibiotics were obtained from the culture filtrate of an actinomycete identified as *Streptomyces violaceolatus*, and were named kerriamycins A, B and C, respectively. The structures of these kerriamycins were elucidated by NMR spectral analysis and chemical degradation, which revealed that each kerriamycin was composed of a common chromophore identified as aquayamycin and two or three mols of hexoses. The kerriamycins inhibited the growth of Gram-positive bacteria and prolonged the survival periods of mice bearing Ehrlich ascites carcinoma.

During the course of our screening program for new antitumor antibiotics, three active substances were obtained from the culture filtrate of a streptomycete, and were named kerriamycins A, B and C, respectively.^{1,2)} On the basis of the structural similarity of the kerriamycins and capoamycin^{3,4)} to tetrangomycin,⁵⁾ aquayamycin,⁶⁾ P-1894B,⁷⁾ the sakyomicins⁸⁾ and the saquayamycins,⁹⁾ etc., which contain the modified benz[a]anthraquinone chromophore in common, we have proposed the generic name "isotetracenone antibiotics" to cover the compounds belonging to this family.

This paper describes the production, isolation, structural elucidation and biological properties of the kerriamycins, as well as the taxonomy of their producing organism, *Streptomyces violaceolatus* A32.

Taxonomy of strain A32

The kerriamycin-producing strain A32 was isolated from a soil sample collected at Yanagawa-shi, Fukuoka, Japan. Characterization of the strain was performed by the method described by Shirling and Gottlieb.¹⁰⁾

The aerial mycelium of the strain mono-

podially branched on the long main stem and terminated in spirals, and occasionally hooks or loops, forming spore chains with 10~50 spores per chain (Fig. 1). The spores were oval to cylindrical ($0.6\sim0.8\times0.9\sim1.1\ \mu\text{m}$) with a smooth surface (Fig. 2).

The cultural and physiological characteristics of strain A32 grown on various media at 27°C for 3 weeks are shown in Tables I and II, respectively. These properties can be summarized as follow: the spore chains were spiral; the spore surface was smooth; the aerial mass color was light olive gray to light brownish gray; the reverse side of the colony was reddish brown or red; the soluble pigments were reddish brown or light brown; melanoid pigments were not produced; all tested sugars were utilized for growth as carbon sources; and LL-diaminopimelic acid was detected in the whole-cell hydrolysate.

On the basis of the preceding characteristics, strain A32 seems to belong to the genus *Streptomyces*. Among the species of *Streptomyces* described in Shirling's reports,¹¹⁾ the properties of strain A32 are in good agreements with those of *Streptomyces violaceolatus*, except for slight differences between

FIG. 1. Spore Chains of Strain A32 on Oatmeal Agar, 1 Week.

FIG. 2. Electron Micrograph of Spores of Strain A32 on Oatmeal Agar, 1 Week.

TABLE I. CULTURAL CHARACTERISTICS OF STRAIN A32

Medium	Growth	Aerial mycelium	Reverse side of colony	Soluble pigment
Sucrose-nitrate agar	Good	Abundant, powdery, light olive gray to yellowish red	Yellowish red to deep red	Slightly, pale brown
Glucose-asparagine agar	Poor	None	Yellowish white	None
Glycerol-asparagine agar (ISP No. 5)	Moderate	Moderate, powdery, light brownish gray to pale yellowish brown	Pale yellow to dark red	None
Inorganic salts-starch agar (ISP No. 4)	Good	Abundant, powdery, light brownish gray	Dark brownish purple	Slightly, reddish brown
Tyrosine agar (ISP No. 7)	Good	Moderate, powdery, light olive gray to pinkish gray	Reddish brown to pale orange	None
Nutrient agar	Moderate	Moderate, powdery, pale pink	Dull orange	None
Yeast extract-malt extract agar (ISP No. 2)	Good	Abundant, powdery, dull yellow orange	Dull orange to dark orange	Light brown
Oatmeal agar (ISP No. 3)	Moderate	Moderate, powdery, light brownish gray	Red	Slightly, yellowish white

TABLE II. PHYSIOLOGICAL CHARACTERISTICS OF STRAIN A32

Melanoid production on		
Tyrosine agar (ISP No. 7)		Negative
Peptone–yeast extract iron agar (ISP No. 6)		Negative
Tryptone–yeast extract broth (ISP No. 1)		Negative
Hydrolysis of		
Starch (Inorganic salts–starch agar, ISP No. 4)		Weakly positive
Gelatin (Gelatin–peptone–gelatin)		Negative
Skim milk		
Coagulation		Negative
Peptonization		Weakly positive
Reduction of nitrate		Positive
Temperature range for growth		20 ~ 37°C
Optimum		27°C
Carbon utilization (Pridham & Gottlieb basal, ISP No. 9)		
D-Glucose	+	
D-Fructose	+	
Inositol	+	
D-Mannitol	+	
L-Arabinose	+	
D-Xylose	+	
L-Rhamnose	+	
Raffinose	+	
Sucrose	+	
Whole cell hydrolysate: LL-diaminopimelic acid		

their reverse side colors and soluble pigments. Therefore, strain A32 was identified as a strain of *Streptomyces violaceolatus*.

Production and isolation of the kerriamycins

Strain A32 was cultured at 27°C for 2 days with shaking in 50-ml tubes containing 15 ml of a medium consisting of 0.4% glucose, 1% malt extract and 0.4% yeast extract (at pH 7.0). Thereafter, 2-ml portions of the culture were inoculated into 500-ml Erlenmeyer flasks containing 100 ml of a medium consisting of 2.5% glucose, 1.5% soybean meal, 0.2% dry yeast and 0.4% calcium carbonate (at pH 7.0), the fermentation being carried out at 27°C on a rotary shaker.

It is noticeable that the time course of kerriamycin A production differed from those of B and C, suggesting the biosynthetic sequence of these antibiotics.

The 4-day-cultured broth (1 l) was filtered through celite, and the filtrate was extracted twice with 500 ml of ethyl acetate. The organic layer was dried over anhydrous sodium sul-

fate, concentrated *in vacuo* and subjected to silica gel column chromatography (40 × 4 cm i.d.). The active fraction was eluted with chloroform–methanol (20:1) and evaporated *in vacuo* to give crude kerriamycin A. This material was applied to a Toyopearl HW40F column (40 × 4 cm i.d.), which was developed with methanol. The active eluate was concentrated to dryness to give a yellow powder of kerriamycin A (160 mg) in pure form.

The 1-day-cultured broth (1 l) was used for isolating kerriamycins B and C. After extracting the broth filtrate twice with 500 ml of ethyl acetate, the solvent layer was dried over anhydrous sodium sulfate and concentrated *in vacuo*. This material was subjected to silica gel column chromatography (40 × 4 cm i.d.) and eluted with chloroform–methanol (10:1). The active fraction was evaporated *in vacuo* to give a mixture of kerriamycins B and C. This mixture was further purified by Toyopearl HW40F column chromatography (40 × 4 cm i.d.). Development of the column with methanol gave two yellow bands, which were

FIG. 3. ^1H NMR Spectrum of Kerriamycin A in CHCl_3 . FIG. 4. ^1H NMR Spectrum of Kerriamycin B in CD_3OD .

FIG. 5. ^1H NMR Spectrum of Kerriamycin C in Methanol- d_4 .

FIG. 6. Structures of Kerriamycins A, B and C.*

* In refs 1 and 2, the structures of the kerriamycins should appear as shown here.

separately collected and concentrated to dryness to give yellow powders of kerriamycins B (300 mg) and C (40 mg).

Structural elucidation

Hydrolysis of each kerriamycin with 2N hydrochloric acid for 2 hours at room temperature gave aquayamycin^{6,7)} as an aglycone

part, which was extracted with ethyl acetate and identified by directly comparing with an authentic sample. Aquayamycin, an antitumor antibiotic, contains a modified benz[a]anthraquinone chromophore, which is characteristic of the isotetracenone antibiotics.

The 400 MHz ^1H NMR spectra of kerriamycins A, B and C are shown in Figs. 3, 4 and 5, respectively.

The ^1H NMR spectrum of kerriamycin A (I) in chloroform- d_1 showed the following signals: δ 12.31 (s, 8-OH), 7.91 (d, $J=7.9$ Hz, 10-H), 7.67 (d, $J=7.9$, 11-H), 6.89 (d, $J=9.9$, 6-H), 6.41 (d, $J=9.9$, 5-H), 4.88 (d, $J=11.0$, 1'-H), 4.35 (br. s, 4'-OH), 3.72 (ddd, $J=10.0$, 8.2, 4.0, 3'-H), 3.50 (dq, $J=8.6$, 6.1, 5'-H), 3.18 (dd, $J=8.6$, 8.2, 4'-H), 2.78 (dd, $J=12.7$, 2.8, 2-Ha), 2.51 (d, $J=12.7$, 2-Hb), 2.46 (d, $J=13.0$, 4.0, 2'-Heq), 2.17 (dd, $J=14.8$, 2.8, 4-Ha), 1.85 (d, $J=14.8$, 4-Hb), 1.43 (3H, d, $J=6.1$, 6'-H), 1.42 (ddd, $J=13.0$, 11.0, 10.0, 2'-Hax) and 1.24 (3H, s, 3-CH₃) ascribed to the aquayamycin moiety (I); δ 5.04 (br. s, 1-H), 4.13 (q, $J=6.8$, 5-H), 3.60 (br. s, 4-H), 2.15 (m, 2-Ha), 2.01 (m, 3-Ha), 1.99 (m, 3-Hb), 1.58 (m, 2-Hb) and 1.21 (3H, d, $J=6.8$, 6-H) due to sugar A (II); δ 5.39 (br. s, 1-H), 3.67 (q, $J=6.8$, 5-H), 3.44 (br. s, 4-H), 1.90 (2H, m, 2-H), 1.89 (m, 3-Ha), 1.72 (m, 3-Hb) and 0.58 (3H, d, $J=6.8$, 6-H) due to sugar B (III); δ 4.67 (dd,

$J=9.1$, 2.5, 1-H), 3.81 (d, $J=9.7$, 4-H), 3.44 (br.s, 4-OH), 3.28 (dq, $J=9.7$, 5.8, 5-H), 2.92 (dd, $J=14.0$, 2.5, 2-Heq), 2.81 (dd, $J=14.0$, 9.1, 2-Hax) and 1.46 (3H, d, $J=5.8$, 6-H) due to sugar C (**IV**), which were assigned by 2-D COSY spectral analysis.

The components **II** and **III** were identified as rhodnose¹²⁾ (2,3,6-trideoxy-*threo*-hexopyranose) by this spectral data. The component **IV** was elucidated to be a 2,6-dideoxyhexopyranose with a quaternary carbon at C-3 by ¹H NMR spectral analysis. The chemical shifts of 2-Hax, 2-Heq and 4-H, and the ketone carbonyl carbon observed at δ c201.1 suggest that **IV** was a 3-ulose. In addition, long-range coupling (1.5 Hz) was observed between 2-Hax and 4-H, which were in a 1,3-diaxial-2-keto system.¹³⁾ From these analyses, the structure of **IV** was established to be 2,6-dideoxy-*erythro*-hexopyran-3-ulose. As far as we know, **IV** is a novel sugar found for the first time from a natural source, and thus, the name kerriose was given.

Based on the coupling constants of the

anomeric protons, the anomeric configurations of the sugar moieties were established to be α for each rhodnose and β for kerriose.

The NOE observed between 1-H of **II** and 3'-H of **I**, and between 1-H of **IV** and 4-H of **II** indicate that the glycosidic linkages between sugar A, C and the aquayamycin moiety were as shown in Fig. 6. Irradiation of H-1 (δ 5.39) of sugar B caused NOE enhancement on the carbon⁷⁾ at δ 80.2 assigned to C-12b, thereby showing that the remaining rhodnose (**III**) was glycosidically connected to C-12b of the

FIG. 7. LSPD Experiments for Kerriamycin A.

TABLE III. ¹³C CHEMICAL SHIFT ASSIGNMENTS OF KERRIAMYCINS A, B AND C (ppm)

	A (CDCl ₂)	B (CD ₃ OD)	C (CD ₃ OD)		A	B	C
				Sugar A			
1	204.9	204.0	206.1	1	96.9	95.2	95.1
2	53.9	54.8	53.2	2	25.1	25.7	25.7
3	75.3	77.0	77.5*	3	24.5	25.5	25.5
3-CH ₃	29.9	30.1	30.3	4	76.4	77.5	77.5
4	43.3	44.5	44.8	5	67.1	67.8	67.7
4a	81.6	82.6	81.9	6	17.1	17.5	17.5
5	144.5	146.0	145.8	Sugar B			
6	116.4	117.6	117.9	1	94.4	95.4	
6a	136.9	138.2	138.7	2	23.1	24.3	
7	187.2	189.0	189.0	3	25.4	26.5	
7a	113.5	115.1	115.1	4	66.8	67.8	
8	157.4	158.2	158.2	5	67.0	68.1	
9	138.1	138.7	139.5	6	16.6	17.0	
10	133.4	134.1	134.0	Sugar C			
11	119.7	120.0	119.7	1	101.6	102.6	102.6
11a	129.8	131.9	131.8	2	46.9	40.7	40.7
12	181.6	183.4	182.9	3	201.1	72.2	72.2
12a	138.5	141.1	140.0	4	78.0	78.3	78.3
12b	80.2	82.4	77.8*	5	72.6	73.1	73.1
				6	18.8	18.5	18.5

* Interchangeable.

aquayamycin part. From these analyses, the structure of **1** was determined to be as shown in Fig. 6.

The ^1H and ^{13}C NMR spectra of kerriamycin B (**2**) in methanol- d_4 showed a close resemblance to those of **1**, except for signals relating to sugar C. The ^1H NMR assignments of sugar C in **2** are as follows: δ 4.57 (dd, $J=9.6$, 1.8 Hz, 1-H), 3.49 (ddd, $J=12.0$, 8.9, 5.3, 3-H), 3.22 (dq, 9.4, 5.9, 5-H), 2.90 (dd, $J=9.4$, 8.9, 4-H), 2.20 (ddd, $J=12.2$, 5.3, 1.8, 2-Heq), 1.55 (ddd, $J=12.2$, 12.0, 9.6, 2-Hax) and 1.26 (3H, d, $J=5.9$, H-6). By these spectral data, sugar C in **2** was identified as olivose¹⁵⁾ (2,6-dideoxy-*arabino*-hexopyranose) with the β -configuration, indicating that kerriamycin B was a derivative of **1** possessing olivose in place of kerriose (Fig. 6).

The ^1H and ^{13}C NMR spectra of kerriamycin C (**3**) in methanol- d_4 revealed that the signals due to sugar B in **2** disappeared in **3**, with the remaining part unchanged. Thus, kerriamycin C was elucidated to be the 12b-*O*-derhodinosyl derivative of **2** (Fig. 6).

The ^{13}C chemical shift assignments of these kerriamycins were achieved by 2-D C-H correlation spectral analysis and long-range selective proton decoupling (LSPD) experiments for kerriamycin A (Fig. 7). The results are summarized in Table III.

In order to establish the absolute configu-

ration, kerriamycin B was hydrolyzed with 2N hydrochloric acid at room temperature for 2 hours to give aquayamycin, rhodinosose and olivose. The specific rotations of olivose ($[\alpha]_D^{21} + 50^\circ$, $c=0.26$, H_2O) and rhodinosose 2,4-dinitrophenylhydrazone ($[\alpha]_D^{22} - 19^\circ$, $c=0.08$, pyridine) reveal that these sugars were D-olivose (lit. $+31^\circ$)¹⁵⁾ and L-rhodinosose (lit.

TABLE IV. ANTITUMOR ACTIVITIES OF KERRIAMYCINS A AND B AGAINST EHRLICH MOUSE ASCITES CARCINOMA

Dose (mg/kg/day)	T/C ^a	50-day survivors ^b
Kerriamycin A		
50	Toxic	0/5
25	70	0/5
12.5	208	0/5
6.25	205	0/5
3.13	>196	1/5
1.56	136	0/6
Kerriamycin B		
100	Toxic	0/5
50	152	0/5
25	133	0/5
12.5	208	0/5

Treatment schedule: days 1, 5 (*i.p.*).

^a The ratio of the mean survival time of the treated group divided by that of the control group.

^b The number of surviving mice on day 50 among the tested mice.

TABLE V. ANTIMICROBIAL SPECTRA OF KERRIAMYCINS A, B AND C (MIC, $\mu\text{g/ml}$)

Organism	A	B	C
<i>Staphylococcus aureus</i> FDA 209P	12.5	12.5	12.5
<i>Bacillus subtilis</i> ATCC 6633	12.5	12.5	25.0
<i>Bacillus cereus</i> IAM 1729	12.5	12.5	12.5
<i>Micrococcus luteus</i> ATCC 9341	6.25	6.25	6.25
<i>Escherichia coli</i> NIHJ JC-2	>100	>100	>100
<i>Klebsiella pneumoniae</i> PCI-602	>100	>100	>100
<i>Salmonella typhimurium</i> IID 971	>100	>100	>100
<i>Serratia marcescens</i> IAM 1184	>100	>100	>100
<i>Pseudomonas aeruginosa</i> NCTC 10490	>100	>100	>100
<i>Saccharomyces cerevisiae</i> ATCC 9763	>100	>100	>100
<i>Candida albicans</i> No. Yu 1200	>100	>100	>100
<i>Aspergillus fumigatus</i> IFO 4400	>100	>100	>100
<i>Penicillium chrysogenum</i> ATCC 10002	>100	>100	>100
<i>Tricophyton mentagrophytes</i>	>100	>100	>100

–14.9),¹²⁾ respectively.

From these experimental results, the structures of kerriamycins A, B and C were established as those shown in Fig. 6.

Biological properties

The effects of kerriamycins A and B on Ehrlich mouse ascites carcinoma are summarized in Table IV. Intraperitoneal injections of the kerriamycins on days 1 and 5 caused prolongation of the life spans of the treated mice.

Kerriamycins A, B and C were active against Gram-positive bacteria, but inactive against Gram-negative bacteria, yeasts and fungi. The minimum inhibitory concentrations of the kerriamycins were as shown in Table V.

EXPERIMENTAL

General. Melting points (mp) were determined by a Yanaco micro-apparatus. Specific rotations were obtained with a JASCO DIP-140 spectropolarimeter at 589.6 nm. Mass spectra were measured on a JEOL DX-300 spectrometer. UV and visible spectra were recorded using a Shimadzu UV-300 spectrophotometer. NMR spectra were obtained on a JEOL GX-400 spectrometer with ¹H NMR at 400 MHz and ¹³C NMR at 100 MHz. Chemical shifts are given in ppm using TMS as an internal standard.

Kerriamycin A (1). mp 177~182°C (dec.); [α]_D²¹ +55° (*c*=0.1, MeOH); FAB-MS *m/z* 865 (*M*+Na)⁺. *Anal.* Found: C, 61.05; H, 6.52; O, 32.43. Calcd. for C₄₃H₅₄O₁₇: C, 61.27; H, 6.46; O, 32.27. UV $\lambda_{\max}$ (MeOH)nm (*E*₁^{1%}_{1cm}) 220 (349), 318 (61), 423 (70); IR $\nu_{\max}$ (KBr)cm⁻¹ 3430, 1725, 1655, 1637.

Kerriamycin B (2). mp 188~193°C (dec.); [α]_D²¹ +39° (*c*=0.1, MeOH); FAB-MS *m/z* 867 (*M*+Na)⁺. *Anal.* Found: C, 61.12; H, 6.84; O, 32.04. Calcd. for C₄₃H₅₄O₁₇: C, 61.13; H, 6.68; O, 32.19. UV $\lambda_{\max}$ (MeOH)nm (*E*₁^{1%}_{1cm}) 220 (345), 319 (58), 422 (70); IR $\nu_{\max}$ (KBr)cm⁻¹ 3440, 1725, 1655, 1637.

Kerriamycin C (3). mp 176~181°C (dec.); [α]_D²¹ +37° (*c*=0.1, MeOH); FAB-MS *m/z* 753 (*M*+Na)⁺. *Anal.* Found: C, 60.84; H, 6.61; O, 32.55. Calcd. for C₃₇H₄₆O₁₅: C, 60.81; H, 6.35; O, 32.84. UV $\lambda_{\max}$ (MeOH)nm (*E*₁^{1%}_{1cm}) 219 (369), 316 (70), 421 (78); IR $\nu_{\max}$ (KBr)cm⁻¹ 3400, 1720, 1655, 1638.

Hydrolysis of kerriamycin B (2). A solution of 2 (100 mg)

in 2 N HCl (15 ml) was stirred for 2 hr at room temperature and then extracted with EtOAc (20 ml × 3). The organic layer was dried over anhydrous sodium sulfate and concentrated to dryness to give a yellow powder of aquayamycin (65 mg). mp 188~193°C (dec.); [α]_D²² +155° (*c*=0.1, dioxane); FAB-MS *m/z* 509 (*M*+Na)⁺; UV $\lambda_{\max}$ (MeOH)nm (*E*₁^{1%}_{1cm}) 220 (590), 316 (98), 421 (119); ¹H NMR (CD₃OD) δ : 7.80 (d, *J*=7.8 Hz, 10-H), 7.52 (d, *J*=7.8, 1-H), 6.83 (d, *J*=9.7, 6-H), 6.39 (d, *J*=9.7, 5-H), 4.83 (dd, *J*=11.0, 1.2, 1'-H), 3.67 (ddd, *J*=10.6, 8.9, 5.0, 3'-H), 3.43 (dq, *J*=9.0, 6.1, 5'-H), 3.02 (dd, *J*=9.0, 8.9, 4'-H), 2.81 (d, *J*=12.7, 2-Hax), 2.66 (dd, *J*=12.7, 2.0, 2-Heq), 2.38 (ddd, *J*=12.7, 5.0, 1.2, 2'-Heq), 2.02 (2H, m, 4-H), 1.36 (3H, d, *J*=6.1, 6'-H), 1.24 (ddd, *J*=12.7, 11.0, 10.6, 2'-Hax), 1.22 (3H, s, 3-CH₃).

Isolation of L-rhodinose 2,4-dinitrophenylhydrazone. The aqueous residue obtained from the preceding EtOAc extraction was extracted with CHCl₃-MeOH (9:1, 20 ml × 3). The organic layer was dried over anhydrous sodium sulfate and concentrated to dryness to give a rhodinose fraction. A solution of this fraction in EtOH (2 ml) was added to a mixture of 2,4-dinitrophenylhydrazine (50 mg), conc. H₂SO₄ (200 mg) and 75% EtOH (2 ml). After stirring for 10 min at room temperature, 50 ml of H₂O was added. The EtOAc extract (20 ml × 3) of the solution was dried over anhydrous sodium sulfate and evaporated *in vacuo*. This material was subjected to silica gel column chromatography (20 × 2 cm i.d.) and eluted with CHCl₃-MeOH (15:1). A phenylhydrazone fraction was collected, and subsequent evaporation to dryness gave a yellow crystalline powder of L-rhodinose 2,4-dinitrophenylhydrazone (8 mg). [α]_D²² -19° (*c*=0.08, pyridine).

Isolation of D-olivose. The aqueous residue obtained from the previous extraction of rhodinose was evaporated *in vacuo* and applied to a silica gel column (20 × 2 cm i.d.). Development of the column with CHCl₃-MeOH (10:1) gave an olivose fraction. This fraction was concentrated to dryness to give a colorless powder of D-olivose (5 mg). [α]_D²¹ +50° (*c*=0.26, H₂O).

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