QUINALDOPEPTIN†, A NOVEL ANTIBIOTIC OF THE QUINOMYCIN FAMILY

SOICHIRO TODA, KOKO SUGAWARA, YUJI NISHIYAMA, MASARU OHBAYASHI,
NORIYUKI OHKUSA, HARUAKI YAMAMOTO, MASATAKA KONISHI
and TOSHIKAZU OKI

Bristol-Myers Research Institute, Ltd., Tokyo Research Center,
2-9-3 Shimo-meguro, Meguro-ku, Tokyo 153, Japan

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Quinaldopeptin, a new type of quinomycin antibiotic, was isolated from the culture of *Streptoverticillium album* strain Q132-6. The antibiotic exhibited strong *in vitro* antimicrobial and cytotoxic activity and significantly prolonged the survival time of mice inoculated with a murine tumor. Quinaldopeptin is a symmetric cyclic peptide linked only by peptide bonds and differs from known antibiotics of the quinomycin family by the lack of ester linkage.

In our search for microbial metabolites with antitumor activity, a novel antibiotic, quinaldopeptin has been isolated from the cultured broth of *Streptoverticillium album* strain Q132-6 which was isolated from a soil sample collected in Andhra Pradesh, India. The antibiotic was recovered from the fermentation broth by solvent extraction and purified by chromatography. Structural studies combining spectral analysis and chemical degradation disclosed that quinaldopeptin is a symmetric cyclic peptide comprised of 2 mol each of 3-hydroxyquinaldic acid, glycine, sarcosine and α,β-diaminobutyric acid and 4 mol of pipecolic acid. Although quinaldopeptin should be classified in the quinomycin group of antibiotics, it distinctly differs from the known compounds by the lack of an ester linkage. Quinaldopeptin is highly active against Gram-positive bacteria and anaerobes and strongly cytotoxic against cultured B16 melanoma cells. It prolonged the survival time of mice transplanted with P388 leukemia. This paper describes the isolation, properties, structural determination and biological activities of quinaldopeptin.

Isolation of the Producing Organism

An actinomycete, strain No. Q132-6, which produces quinaldopeptin along with aureothricin and a methylpentaene, was isolated from a soil sample collected in Andhra Pradesh State, India. Strain Q132-6 was identified as genus *Streptoverticillium*, and classified into *S. album*.

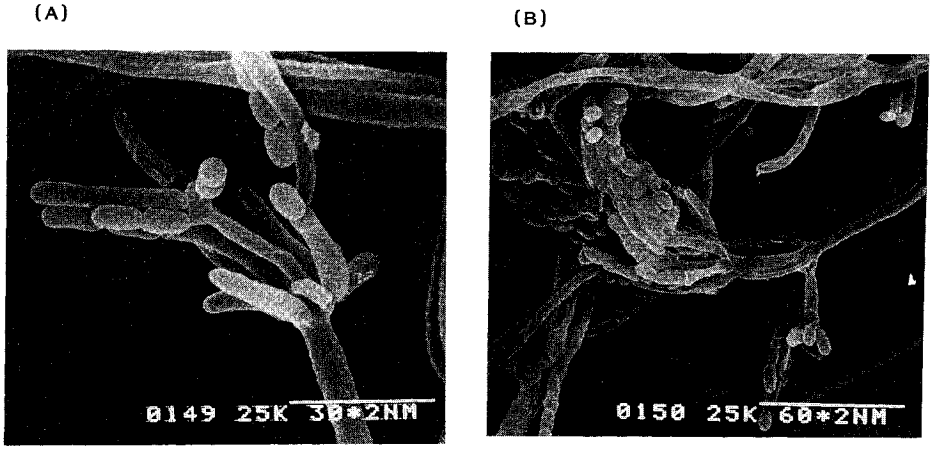
Taxonomy of the Producing Organism

Morphology

Substrate mycelium is well-branched and appears non-fragmented in the intact colony. The formation of aerial mycelium is very poor. Short straight spore chains are borne on the biverticillate sporophores. The sporulation is limited to CZAPEK's sucrose-nitrate agar and ISP medium No. 9, supplemented with D-mannitol or some other sugar. Irregular tufty sporophores or verticils with scanty spores are often observed (Fig. 1). The spore chains contain 2 to 10 spores per sporophore; the spores are oval or short-cylindrical ($0.6 \times 0.8 \sim 1.5\ \mu m$), and have a smooth surface. A balloon-like body is occasionally formed, but motile spores, sporangia or true sclerotia were not observed.

† Quinaldopeptin was originally called as BMY-28662 or BU-3845T.

Fig. 1. Scanning electron micrographies of *Streptoverticillium album* strain Q132-6.
(A) Immature verticillate sporophores, (B) mature biverticillate sporophores.



Medium: CZAPEK's sucrose-nitrate agar, cultivation: 28°C for 1 month.

Table 1. Cultural characteristics of strain Q132-6.

Medium	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigment
Sucrose-nitrate agar (CZAPEK-Dox agar)	Poor	Very scant; white (263)	Colorless	None
Tryptone-yeast extract broth (ISP No. 1)	Moderate; not turbid	None	Colorless	None
Yeast extract-malt extract agar (ISP No. 2)	Good	None	Moderate orange yellow (71)	None
Oatmeal agar (ISP No. 3)	Moderate	None	Light yellowish brown (76)	Grayish greenish yellow (105)
Inorganic salts-starch agar (ISP No. 4)	Moderate	Scant; white (263)	Light grayish yellowish brown (79)	Brilliant greenish yellow (98)
Glycerol-asparagine agar (ISP No. 5)	Moderate	Very scant; white (263)	Pale yellow (89)	Light yellow (86)
Peptone-yeast extract-iron agar (ISP No. 6)	Good	None	Grayish yellow (90)	None
Tyrosine agar (ISP No. 7)	Moderate	Very scant; white (263)	Light grayish yellowish brown (79)	Brilliant yellow (83)
Glucose-asparagine agar	Poor	Very scant; white (263)	Light yellowish brown (76)	None
Nutrient agar	Good	None	Dark grayish yellow (91)	None
BENNETT's agar	Good	Very scant; white (263)	Moderate orange yellow (71)	None

Observation after incubation at 28°C for 3 weeks.
Color name used: ISCC-NBS Color-Name Charts.

Cultural and Physiological Characteristics

Color of aerial mycelium is white, or occasionally white with yellow to grayish shades after long incubation. Yellowish diffusible pigment is produced in ISP media Nos. 3, 4, 5 and 7. Melanin and other distinct pigments are not produced (Table 1). The physiological characteristics of strain Q132-6 are shown in Table 2.

Table 2. Physiological characteristics of strain Q132-6.

Temperature:		Salicin	−
Growth range	20~48°C	Production of:	
Optimal growth	30~44°C	Melanin	−
No growth	17 and 50°C	Utilization of:	
Tolerance to:		Coumarin	−
Lysozyme, 0.01% (w/v)	−	L-Methionine	+
NaCl 1~6% (w/v)	+	L-Proline	+
8% (w/v) or more	−	Shikimic acid	+(w)
pH 4.5~10.5	+	DL-Aminobutyric acid	−
Production of:		Acid production from:	
Gelatinase	+	D-Galactose	+(w)
Amylase	+	Inositol	+
Nitrate reductase	−	D-Fructose	+
Tyrosinase	−	D-Ribose	+
Utilization of:		D-Trehalose	+(w)
Glycerol	+(w)	Degradation of:	
D-Arabinose	−	Aesculin	+
L-Arabinose	−	Citrate	−
D-Xylose	−	DNA	+
D-Ribose	+	Hypoxanthine	+
L-Rhamnose	−	L-Tyrosine	+
D-Glucose	+	Tween 20	+
D-Galactose	±	Production of:	
D-Fructose	+	H_2S	−
D-Mannose	+(w)	Growth with:	
L-Sorbose	−	1-Phenolethanol	+
Sucrose	±	Potassium tellurite	+
Lactose	−	Crystal violet	−
Cellobiose	+	Malachite green	+
Melibiose	−	Resistant to:	
Trehalose	+(w)	Carbenicillin	+
Raffinose	−	Cephaloridine	+
D-Melezitose	−	Cephalothin	+
Soluble starch	+	Cefamandole	+
Cellulose	−	Colistin	−
Dulcitol	−	Antibiosis to:	
Inositol	+	*Aspergillus niger*	+
D-Mannitol	+(w)	*Bacillus subtilis*	+
S-Sorbitol	±	*Candida albicans*	−

+: Positive, +(w): weakly positive, ±: marginal, −: negative.

Chemotaxonomy

Whole-cell hydrolysate contains LL-diaminopimelic acid, ribose and glucose, and hence belongs to cell wall type I_{NC}. The phospholipid contains phosphatidylethanolamine, hence is placed in type P-II.

Taxonomic Position

The above-mentioned morphology and chemotaxonomy indicate that strain Q132-6 belongs in the genus *Streptoverticillium*. According to the descriptions of the species by BALDACCI and LOCCI[1], SHIRLING and GOTTLIEB[2], and WILLIAMS *et al.* [3], it is similar to *Streptoverticillium ladakanum*, *Streptoverticillium morookaensis* and *S. album*. Physiologically, Q132-6 differs slightly from the three species (Table 3), but it is similar to *S. album* in aureothricin production and cultural characteristics. Thus, strain Q132-6 was designated *S. album*.

Antibiotic Production

A loopful of mature slant culture of *S. album* strain No. Q132-6 was inoculated into a seed medium (100 ml) composed of lactose 1%, soluble starch 3% (Nichiden Kagaku), fish meal 1% (Hokuyo Suisan), $CaSO_4 \cdot 2H_2O$ 0.6% and $CaCO_3$ 0.5% (pH 7.0) in a 500-ml Erlenmeyer flask, and was incubated at 28°C for 4 days on a rotary shaker at 200 rpm. Five ml of the culture was transferred into a 500-ml Erlenmeyer flask containing 100 ml of the production medium having the same composition as the seed medium. The fermentation was carried out at 28°C for 5 days on a rotary shaker at 200 rpm. The fermentation study was also carried out in a jar fermenter. Five hundred ml of the seed culture prepared by the above method was transferred into a 20-liter jar fermenter containing 12 liters of the production medium as described above. The jar fermenter was aerated at the rate of 12 liters per minute and agitated at 250 rpm at 28°C. The antibiotic activity in the fermentation broth was monitored by the *in vitro* cytotoxic activity against cultured B16 melanoma cells using a microtiter plate. The harvested broth after 70-hour fermentation showed a potency of approximately 100 μg/ml.

Table 3. Differential characteristics of strain Q132-6 and three relevant species of *Streptoverticillium*.

	Strain Q132-6	*S.l.*	*S.m.*	*S.a.*
Production of:				
Melanin	−	−	−	−
Other pigments	−	−	−	−
Utilization of:				
D-Mannitol	+(w)	−	+	−
Inositol	+	−	+	−
Trehalose	+(w)	−	+	+
Degradation of:				
Citrate	−	−	+	+
DNA	+	+	−	+
Hypoxanthine	+	→	−	−
Growth with:				
NaCl, 5% (w/v)	+	−	+	−
Crystal violet, 0.01% (w/v)	−	−	+	−

Abbreviations: *S.l.*, *Streptoverticillium ladakanum*; *S.m.*, *S. morookaensis*; *S.a.*, *S. album*. +, positive; +(w), weakly positive; −, negative.

Isolation and Purification

The harvested broth (19 liters) was extracted with 16 liters of butanol at pH 7.6. The extract was concentrated under reduced pressure and applied to a column of silica gel (Wako gel C-200, i.d. 4.0 × 60 cm). The column was developed with a mixture of methanol and ethyl acetate with a stepwise increasing methanol concentration (0→20%). The eluate was monitored by the bioassay using *Bacillus subtilis* PCI 219 and by spot test of UV absorption on TLC plates. The first bioactive, UV-absorbing fractions were eluted with ethyl acetate. Concentration of the pooled fractions afforded a yellow solid (895 mg) which was identified as aureothricin by its spectral data. The second active fractions eluted with 10% methanol were pooled and concentrated to give a semi-pure solid of quinaldopeptin (13.5 g). The solid was rechromatographed on a silica gel column (i.d. 4.0 × 20 cm) using a mixture of methanol and methylene chloride (methanol concentration 0→5%). After monitoring by bioassay and TLC (SiO_2, chloroform - methanol, 10 : 1), the appropriate fractions were combined and evaporated. The residue was crystallized from a mixture of chloroform and ether to give colorless rods of pure quinaldopeptin (1.38 g).

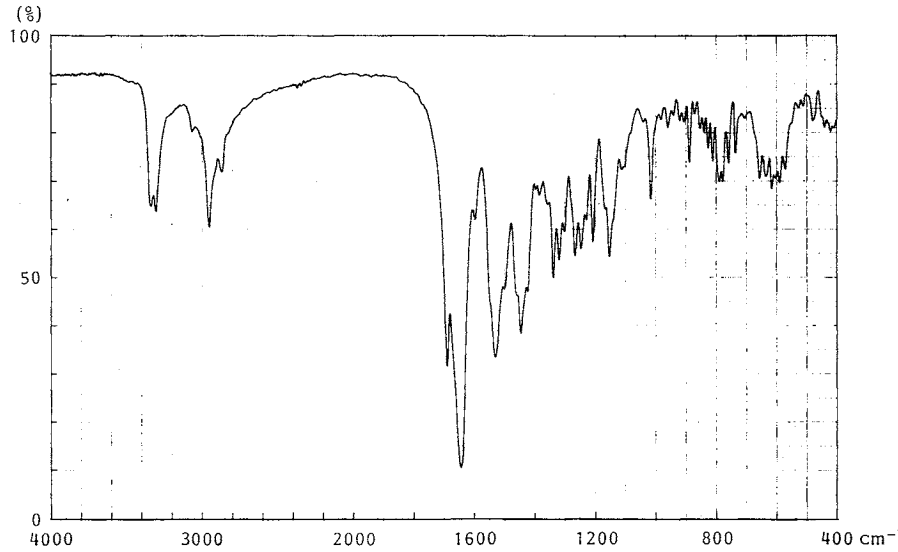
Physico-chemical Properties

Physico-chemical properties of quinaldopeptin are summarized in Table 4. It is soluble in pyridine and slightly soluble in dimethyl sulfoxide but insoluble in other organic solvents and water. This antibiotic showed a positive response to ferric chloride, but negative response to ninhydrin, anthrone and Sakaguchi reactions. The UV spectrum exhibited maxima at 214, 230, 299 and 359 nm in neutral and acidic solution and at 211 (sh), 222, 251, 307 (sh) and 396 nm in alkaline solution. The IR spectrum (Fig. 2) had strong

Table 4. Physico-chemical properties of quinaldopeptin and its acetate.

	Quinaldopeptin	Diacetylquinaldopeptin
Appearance	Colorless rods	Colorless needles
MP (°C)	>300	>300
$[\alpha]_D^{24}$	51±2 (*c* 0.5, pyridine)	
Microanalysis		
Calcd for	$C_{62}H_{78}N_{14}O_{14}\cdot H_2O$:	$C_{66}H_{82}N_{14}O_{16}\cdot 2H_2O$:
C	59.04	58.14
H	6.39	6.36
N	15.55	14.38
Found: C	59.33	58.32
H	6.40	6.22
N	15.36	14.07
SI-MS (*m/z*)	1,266 $(M+Na)^+$	1,350 $(M+Na)^+$
	1,244 $(M+H)^+$	1,328 $(M+H)^+$
UV λ_{max}^{MeOH} nm (ε)	214 (78,000), 230 (73,000), 299 (9,700), 359 (9,300)	208 (80,000), 237 (76,000), 394 (7,200)

Fig. 2. IR spectrum of quinaldopeptin (KBr).



absorption bands at 3340, 3300 (OH and/or NH), 1690, 1645 and 1530 cm^{-1} (amide). The molecular formula was determined to be $C_{62}H_{78}N_{14}O_{14}$ on the basis of the microanalysis and SI-MS data (*m/z* 1,266 $(M+Na)^+$, 1,244 $(M+H)^+$). Acetylation of quinaldopeptin with acetic anhydride and pyridine gave a diacetyl derivative with improved solubility, whose physico-chemical data are also shown in Table 3. The SI-MS spectrum of diacetylquinaldopeptin gave *quasi*-molecular ions at *m/z* 1,350 $(M+Na)^+$ and 1,328 $(M+H)^+$, which accounted for introduction of two acetyl groups.

Structural Studies

Quinaldopeptin did not give clear signals in ^1H or ^{13}C NMR spectra due to its extremely poor solubility in the NMR solvents. The diacetyl derivative showed much improved solubility and the ^{13}C NMR spectrum of the acetate exhibited a total of 33-carbons as summarized in Table 5. They were resolved as three methyl, ten methylene, four methine, nine sp^2 (five with proton and four without proton) and seven carbonyl carbons. Taking into consideration the molecular formula ($C_{66}H_{82}N_{14}O_{16}$) determined as

Table 5. ^{13}C NMR of diacetylquinaldopeptin.

Carbon No.	δ ppm (DMSO-d_6)	Assignment	Carbon No.	δ ppm (DMSO-d_6)	Assignment
1	170.64	Carbonyl	18	52.72	DAB-β
2	170.07	Carbonly	19	50.88	PiP-α
3	169.94	Carbonly	20	50.56	Gly-α
4	169.79	Carbonly	21	48.13	Pip-α'
5	169.47	Carbonly	22	40.48[a]	Sar-α
6	168.80	Carbonly	23	40.38[a]	Pip-ε
7	162.41	Carbonly	24	43.06[a]	Pip-ε'
8	143.84	HQA C-3	25	35.57	NMe
9	143.51	HQA C-2	26	26.16[b]	Pip-β
10	142.57	HQA C-8a	27	26.58[b]	Pip-β'
11	131.25	HQA C-4a	28	25.96	Pip-δ
12	130.94	HQA C-8	29	24.00	Pip-δ'
13	129.93	HQA C-7	30	21.04	Pip-γ
14	129.48	HQA C-5	31	20.67	Pip-γ'
15	129.42	HQA C-6	32	20.57	Acetyl-Me
16	127.71	HQA C-4	33	12.78	DAB-Me
17	55.50	DAB-α			

[a,b] The signals may be reversed.
DAB: α,β-Diaminobutyric acid, Pip: pipecolic acid, HQA: 3-hydroxyquinaldic acid.

discussed above, the ^{13}C NMR of diacetylquinaldopeptin suggested that the antibiotic consisted of two equivalent molecules.

The UV spectrum of quinaldopeptin was almost identical to that of sandramycin[4] indicating the same chromophore, 3-hydroxyquinaldic acid. The IR spectrum, however, lacked the ester absorption at $1730\sim1710\,cm^{-1}$ which all other known echinomycin group antibiotics[5~8] have.

Complete Acid Hydrolysis

Quinaldopeptin was hydrolyzed with 6 N HCl at $110\sim120°C$ for 18 hours in a sealed tube. A yellow crystalline product (**I**) was collected by filtration. The IR and UV spectra and mp of **I** were identical to those reported for 3-hydroxyquinaldic acid[9]. The filtrate was concentrated *in vacuo* and the residue was chromatographed on a column of Dowex 50WX8 (H^+ type) with hydrochloric acid elution. Four amino acids, glycine (**II**), sarcosine (**III**), pipecolic acid (**IV**) and α,β-diaminobutyric acid (**V**) were isolated in a ratio of 1:1:2:1. The L-configuration was assigned to the pipecolic acid by its optical rotational value ($[\alpha]_D$ $-9.9°$)[10]. The NMR spectrum of the α,β-diaminobutyric acid indicated that it was a mixture of the *erythro* and *threo* isomers. It is well recognized that α-amino group of α,β-diaminobutyric acid is readily racemized in acid hydrolysis[11]. Therefore, quinaldopeptin is considered to have α,β-diaminobutyric acid of single absolute configuration which racemized during the acid hydrolysis. Elucidation of the absolute configuration was performed by following reactions[12]. The α,β-diaminobutyric acid obtained above was treated with hydrogen peroxide in the presence of ferrous sulfate to give alanine which was determined to be of "D" configuration by HPLC using a chiral column. Therefore, the β-carbon of the amino acid has *R*-configuration. For determination of the α-carbon configuration, compound **VI**, a partial hydrolysis product as will be discussed later, was deaminated with sodium nitrate under acidic condition. The product was then hydrolyzed to give a mixture of D-threonine and D-allo-threonine by chiral HPLC. Consequently, the absolute configuration of α,β-diaminobutyric acid of quinaldopeptin is D-*erythro*.

Partial Degradation and Determination of the Amino Acid Sequence

Quinaldopeptin was hydrolyzed with 3 N HCl at 70°C for 18 hours. Six degradation products (**VI**, **VII**, **VIII**, **IX**, **X** and **XI**) retaining the UV spectrum of the parent antibiotic were isolated after chromatographic separation. Structure elucidation of these products was performed by further degradations and spectral analysis.

Compound **VI** (*m/z* 290 $(M+H)^+$) produced **I** and **V** upon acid hydrolysis. In the ^1H NMR, the amide NH of **VI** (δ 9.37, d, $J=8.1$ Hz) coupled with α-CH of **V** (δ 4.20) allowing the structure N^{α}-(3-hydroxyquinaldyl)-α,β-diaminobutyric acid to be assigned to this compound. The mass spectrum (*m/z* 401 $(M+H)^+$) and acid hydrolysis (**I**, **IV** and **V**) suggested 1 mol of **IV** bonded to **VI** in compound **VII**. The site of linkage was assigned to the β-amino (δ 8.30, d, $J=8.5$ Hz) of **V** by the ^1H-^1H COSY spectrum. Compound **VIII** ($C_{23}H_{29}N_5O_6$, *m/z* 472 $(M+H)^+$) was similar to **VII** in spectral properties but, upon hydrolysis, produced amino acid **III** in addition to the three constituents of **VII**. In the ^1H NMR spectrum of **VIII**, the α-H of **IV** moiety was observed at δ 4.99, *ca.* 1.3 ppm lower field than the corresponding proton of **VII**. This indicated that unlike that of **VI** and **VII**, the α-H of **IV** of **VIII** took the equatorial conformation upon the formation of the peptide linkage at the NH of **IV** with **III**. The conformational change and resulting chemical shift change of the α-H of pipecolic acid are well exemplified in the structural study of rapamycin[13].

The ^{13}C NMR and mass spectra and complete hydrolysis established the molecular formula and constituents of compounds **IX**, ($C_{22}H_{27}N_5O_6$, **I**, **II**, **IV** and **V**), **X** ($C_{28}H_{36}N_6O_7$, **I**, **II**, 2×**IV** and **V**) and **XI** ($C_{31}H_{41}N_7O_8$, **I**, **II**, **III**, 2×**IV** and **V**). Compound **XI** represented exactly half of the parent antibiotic. The above constituent analysis indicated that **IX**, **X** and **XI** corresponded to **VI**, **VII** and **VIII**, respectively, but possessed an additional 1 mol unit of **II** and **IV**. Therefore, **II** and **IV** should be linked through the carboxylic acid of **V** and the ^1H NMR of the corresponding protons supported this assignment. In order to determine the *C*-terminal amino acid, **XI** was *N*-acetylated and then converted to the methyl ester (*m/z* 710, $(M+H)^+$) which was treated with $LiBH_4$[14]. Acid hydrolysis of the reduction product gave **I**, **III**, **IV** and **V** but no trace of **II**. Thus, the structures of **VI**, **VII**, **VIII**, **IX**, **X** and **XI** were as shown in Fig. 3 which naturally established the structure of quinaldopeptin as Fig. 4.

Fig. 3. Structures of partial hydrolysis products.

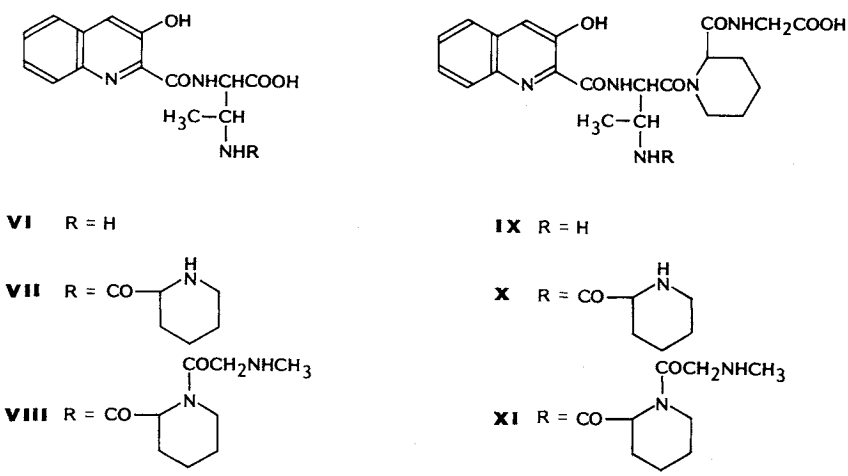


Fig. 4. Structure of quinaldopeptin.

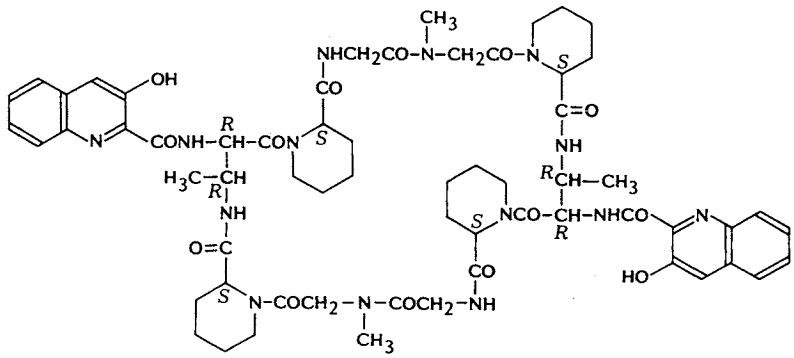


Table 6. Antimicrobial activity.

Organism	Test medium[a]	MIC (μg/ml)	
		Quinaldopeptin	Diacetylquinaldopeptin
Escherichia coli NIHJ	NA	3.1	3.1
Klebsiella pneumoniae D11	NA	6.3	6.3
Pseudomonas aeruginosa A9930	NA	100	12.5
Proteus vulgalis A9436	NA	100	100
Staphylococcus aureus FDA 209P	NA	0.4	0.8
S. aureus Smith	NA	0.4	0.4
S. epidermidis D153	NA	0.4	0.4
Streptococcus faecalis A9612	NA	1.6	3.1
S. pyogenes A20201	NA	0.2	—
Micrococcus luteus PCI 1001	NA	0.2	0.4
Bacillus subtilis PCI 219	NA	0.4	0.8
Bacteroides fragilis A22693	GAM	1.6	1.6
Clostridium difficile A21675	GAM	0.4	<0.2
C. perfringens A9635	GAM	0.8	<0.2
Propionibacterium acnes A21993	GAM	0.4	<0.2
Candida albicans IAM 4888	SDA	>100	>100
Cryptococcus neoformans D49	SDA	3.1	6.3
Aspergillus fumigatus IAM 2530	SDA	>100	100
Trichophyton mentagrophytes D155	SDA	>100	>100

[a] NA: Nutrient Agar (Eiken), GAM: Gifu Anaerobic Medium (Nissui), SDA: Sabouraud dextrose agar.

Antimicrobial Activity

The antimicrobial activity of quinaldopeptin was determined by the 2-fold agar dilution method using Nutrient Agar (Eiken) for Gram-positive and Gram-negative bacteria, Gifu Anaerobic Medium (Nissui) for anaerobic bacteria and Sabouraud dextrose agar for fungi. The antimicrobial spectra of quinaldopeptin and its acetate are shown in Table 6. Both compounds showed strong activity against aerobic Gram-positive bacteria as well as anaerobic Gram-positive bacteria, but they were less active against aerobic Gram-negative bacteria. Antifungal activity of both antibiotics was rather weak except for *Cryptococcus neoformans*.

Antitumor Activity

Quinaldopeptin and diacetylquinaldopeptin were tested for *in vitro* cytotoxicity against murine and human tumor cells and for *in vivo* antitumor activity in mice. Chromomycin A_3 (*in vitro*) or mitomycin C (*in vivo*) was used as a reference compound.

B16-F10 (murine melanoma) and Moser (human colorectal carcinoma) cells were grown in EAGLE's minimum essential medium supplemented with fetal calf serum (10%) and kanamycin (60 μg/ml). Exponentially growing cells were harvested, counted and suspended in the culture medium at 2×10^4 and 5×10^4 cells/ml, respectively. After planting 150 μl of cell suspension into wells of a 96-well microtiter plate with test materials (50 μl), the plates were incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air for 72 hours. The cytotoxicity against the tumor cells was colorimetrically determined at 540 nm after staining viable cells with neutral red solution. As shown in Table 7, quinaldopeptin and diacetylquinaldopeptin were quite active against B16-F10 cells with IC_{50} values approximately 3-fold superior to that of chromomycin A_3. On the other hand, they showed almost the same cytotoxicity as chromomycin A_3 against Moser cells.

The *in vivo* antitumor activity of quinaldopeptin was examined against lymphocytic leukemia P388 in mice. P388 Leukemia was inoculated intraperitoneally into female CDF_1 mice at 10^6 cells per mouse and graded doses of test materials were administered intraperitoneally on days 1 to 3 (Q1D × 3) or on days 1, 5 and 9 (Q4D × 3) after tumor implantation. Death or survival of the treated and non-treated animals was recorded daily during the observation period of 45 days and the median survival time (MST) was calculated for the test (T) and control (C) groups. A T/C value $\geqq$125% is considered a significant antitumor effect. Quinaldopeptin and diacetylquinaldopeptin showed slight prolongation of life-span in P388-bearing mice at 0.03~0.01 mg/kg as shown in Table 8, but the antitumor activities were quite limited.

Table 7. *In vitro* cytotoxicity against murine and human tumor cells.

Compound	IC_{50} (μg/ml)	
	B16-F10	Moser
Quinaldopeptin	0.0008	0.04
Diacetylquinaldopeptin	0.0007	0.04
Chromomycin A_3	0.002	0.05

Table 8. Antitumor activity against P388 leukemia (ip).

Compound	Dose[a] (mg/kg/day)	MST (day)	T/C (%)	Average weight change on day 4 (g)
Expt 1				
Quinaldopeptin	0.03	15.5	**141**[b]	+0.3
	0.01	13.5	123	+1.5
	0.003	12.5	114	+1.5
Mitomycin C	3	22.0	**200**	−0.5
	1	17.0	**155**	−0.3
	0.3	16.5	**150**	+1.0
Vehicle	—	11.0	—	+1.5
Expt 2				
Quinaldopeptin	0.03	Toxic	Toxic	—
	0.01	13.0	**130**	+2.0
	0.003	11.5	115	+2.3
Diacetylquinaldopeptin	0.1	Toxic	Toxic	—
	0.03	13.0	**130**	−0.3
	0.01	12.0	120	+1.0
	0.003	10.0	100	+1.3
Vehicle	—	10.0	—	+0.5

[a] Q1D × 3, ip.

[b] Boldface type indicates significant antitumor effect (T/C$\geqq$125%).

Discussion

Quinaldopeptin is a novel antitumor antibiotic produced by *S. album* No. Q132-6. It is active against a variety of Gram-positive and Gram-negative bacteria, *C. neoformans* and various tumor cells and exerts an antitumor effect against P388 leukemia.

The structural studies disclosed that quinaldopeptin is a symmetric, cyclic chromopeptide similar to the equinomycin-luzopeptin group antibiotics. Since it contains 3-hydroxyquinaldic acid and pipecolic acid, quinaldopeptin is closely related to the luzopeptins[5,15] and sandramycin[4]. However, the amino acid units of quinaldopeptin are linked only by peptide linkages, which distinguishes it from all previously described equinomycin-luzopeptin group antibiotics. These known antibiotics contain two D-serines which form two ester linkages in the core peptide rings. Quinaldopeptin contains two D-*erythro*-α,β-diaminobutyric acids in place of the D-serine of the known antibiotics.

Experimental

General

MP's were determined on a Shibayama micro melting point apparatus and were not corrected. TLC was performed on silica gel plates (Kiesel gel $60F_{254}$, Merck). The IR and UV spectra were recorded on a Jasco IR-810 IR spectrophotometer and a Jasco UVIDEC-610C spectrometer, respectively. The SI-MS and FAB-MS spectra were measured on a Hitachi M80B and Jeol JMS-AX505H mass spectrometer. The ^1H and ^{13}C NMR spectra were recorded on a Jeol JMN-GX 400 spectrometer or Varian FT80A spectrometer. Amino acid analysis was carried out using the Waters PICO-TAG system.

Acetylation

A mixture of quinaldopeptin (51 mg), acetic anhydride (0.25 ml) and dry pyridine (1 ml) was stirred at room temperature for 24 hours. The reaction mixture was diluted with ethyl acetate (30 ml) and then successively washed with dilute hydrochloric acid, water and saturated sodium chloride. The organic layer was dried over Na_2SO_4 and evaporated *in vacuo* to give 60 mg of white powder which was purified by Sephadex LH-20 column chlormatography (2×60 cm) using methanol as the eluant to give the pure diacetate (51 mg).

Complete Acid Hydrolysis of Quinaldopeptin

A suspension of quinaldopeptin (490 mg) in 6 N HCl (20 ml) was heated at 110~120°C for 18 hours in a sealed tube. After cooling, the reaction mixture was filtered to give 139 mg of pale yellow crystalline powder of **I**.

I: Pale yellow crystalline powder; mp 195~197°C (literature 196~198°C)[9], IR ν_{max}^{KBr} cm^{-1} 3430, 2900 and 1688. ^1H NMR (80 MHz, DMSO-d_6) δ 8.09 (1H, d, J=8.0 Hz), 7.96 (1H, s), 7.90 (1H, d, J=8.0 Hz), 7.60 (2H, m). The filtrate was diluted with water and extracted with butanol; the extract was concentrated to give 7 mg of another crop of 3-hydroxyquinaldic acid. The aqueous solution was concentrated to dryness under reduced pressure, and the residue chromatographed on a column of Dowex 50WX8 (H^+ form 25 ml), using an increasing concentration of HCl (0.1~3 N) as eluent. The eluate was monitored by ninhydrin test and TLC (BuOH - AcOH - H_2O, 3 : 1 : 1). Ninhydrin-positive fractions containing the same amino acid as monitored by TLC were combined and evaporated *in vacuo* to yield **II** (glycine, 100 mg), **III** (sarcosine, 110 mg), **IV** (L-pipecolic acid, 256 mg) and **V** (α,β-diaminobutyric acid, 154 mg) as hydrochlorides.

II: Colorless powder, TLC: Rf 0.25 (BuOH - AcOH - H_2O, 3 : 1 : 1) and 0.15 (phenol - H_2O, 4 : 1). Identified as glycine by amino acid analysis, TLC and NMR.

III: Colorless powder, TLC: Rf 0.20 (BuOH - AcOH - H_2O, 3 : 1 : 1) and 0.30 (phenol - H_2O, 4 : 1). Identified as sarcosine by amino acid analysis, TLC and NMR.

IV: Colorless needles from aqueous acetone; mp 258~260°C $[\alpha]_D^{26}$ −9.9° (*c* 2.5, H_2O). TLC: Rf 0.30 (BuOH - AcOH - H_2O, 3 : 1 : 1) and 0.45 (phenol - H_2O, 4 : 1). Identified as L-pipecolic acid hydrochloride by TLC, NMR, mp and $[\alpha]_D$. (Literature mp 258~259°C, $[\alpha]_D$ −10.5° (*c* 9.8, H_2O))[10].

V: Colorless sticky solid, TLC: Rf 0.13 (BuOH - AcOH - H_2O, 3 : 1 : 1) and 0.03 (phenol - H_2O, 4 : 1).

^{1}H NMR (80 MHz, D_2O) δ 1.45 (1.5H, d, J=7.0 Hz), 1.53 (1.5H, d, J=7.5 Hz), 4.0 (1H, m), 4.17 (0.5H, d, J=7.0 Hz), 4.33 (0.5H, d, J=3.5 Hz). HRFAB-MS (Positive) m/z 119.0805 (Calcd as $C_4H_{11}N_2O_4$, 119.0820). Identified as α,β-diaminobutyric acid by NMR and MS.

The Configuration of α,β-Diaminobutyric Acid

A mixture of α,β-diaminobutyric acid (13.2 mg), 3% H_2O_2 (0.2 ml) and $FeSO_4$ (0.2 mg) was heated at 50°C for 1 hour. The reaction mixture was diluted with water and passed through a column of Amberlite IR-120 (H^+ form). The column was eluted with 1 N NH_4OH. The eluate was monitored by TLC (BuOH - AcOH - H_2O, 3 : 1 : 1). The fractions containing alanine were combined and evaporated to dryness to give D-alanine, which was determined by chiral HPLC (column: MCI-gel ODS IHV, 4.6 × 150 mm, Mitsubishi Chemical Industries Limited, mobil phase: 2 mM *N,N*-Dipropyl-L-alanine, 1 mM copper acetate, pH 5.7, flow rate: 0.8 ml/minute, detection: UV 230 nm L-Ala 6.60 minutes, D-Ala 4.73 minutes). A mixture of **VI** (1 mg), 80% aqueous AcOH (0.1 ml), 3 N HCl (0.1 ml) and $NaNO_2$ (2 mg) was stirred at room temperature for 5 hours. After dilution with water, the reaction mixture was extracted with BuOH. The extract was evaporated *in vacuo* to give a yellow residue, which was hydrolyzed with 6 N HCl at 110°C in a sealed tube to give a mixture of D-allo-threonine and D-threonine, determined by TLC (Rf 0.25, BuOH - AcOH - H_2O, 3 : 1 : 1), amino acid analysis and chiral HPLC (column: TSK gel Enantio L1 NoENL 1E 0017, mobile phase: 1 mM $CuSO_4$, flow rate: 1 ml/minute, detection: UV 254 nm, temperature 50°C, D-allo-Thr 4.23 minutes, D-Thr 4.54 minutes, L-allo-Thr 6.19 minutes, L-Thr 5.47 minutes).

Partial Acid Hydrolysis

A suspension of quinaldopeptin (500 mg) in 3 N HCl (40 ml) was heated at 70°C for 18 hours in a sealed tube. The reaction mixture was extracted with BuOH and the extract was concentrated *in vacuo* to give 430 mg of sticky pale yellow residue. The residue was chromatographed on a silica gel column (2.1 × 50 cm) developed with saturated aqueous BuOH to obtain three UV-absorbing fractions. The first fraction containing **VI** as a major component was concentrated and rechromatographed on silica gel using $CHCl_3$ - MeOH (9 : 1). Homogeneous **VI** thus obtained was crystallized from water to give **VI** (14.8 mg). The next fraction containing **VII** and **XI** was chromatographed on a reversed phase silica gel RP18 column (2.1 × 27 cm) eluted with aqueous MeOH with stepwise-increasing MeOH concentration (40%→80%). Compounds **VII** and **XI** were further purified by Sephadex LH-20 (2.1 × 60 cm) chromatography with MeOH as eluent to obtain pure **VII** (7 mg) and **XI** (13.8 mg). The third fraction containing **VIII**, **IX** and **X** was purified by column chromatography on reversed phase silica gel RP18 (2.1 × 30 cm) using a mixture of CH_3CN and 0.015 M phosphate buffer pH 3.5 (CH_3CN 20%→40%). The separated **VIII**, **IX** and **X** were chromatographed on Sephadex LH-20 (2.1 × 60 cm) using MeOH as eluent to give pure **VIII** (28 mg), **IX** (5.6 mg) and **X** (11.9 mg).

VI: MP 190~193°C, SI-MS m/z 290 $(M+H)^+$; UV λ_{max}^{MeOH} nm 220 (ε 29,700), 231 (34,700), 299 (5,010), 358 (4,700); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.19 (3H, d, J=6.8 Hz), 3.61 (1H, m), 4.20 (1H, m), 7.64 (2H, m), 7.86 (1H, s), 7.90 (1H, d, J=7.3 Hz), 8.01 (1H, d, J=7.3 Hz), 8.44 (2H, br), 9.37 (1H, d, J=8.1 Hz), 12.20 (1H, br).

VII: MP 182~186°C, SI-MS m/z 401 $(M+H)^+$; UV λ_{max}^{MeOH} nm 220 (ε 30,600), 230 (33,600), 299 (4,200), 357 (4,240); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.14 (3H, d, J=6.9 Hz), 1.23~1.65 (4H, m), 1.71 (1H, br d, J=12.5 Hz), 2.04 (1H, br d, J=11.3 Hz), 2.90 (1H, dt, J=12.5 and 3.6 Hz), 3.17 (1H, d, J=12.5 Hz), 3.67 (1H, dd, J=11.7 and 2.8 Hz), 4.47 (1H, m), 4.49 (1H, m), 7.62 (2H, m), 7.81 (1H, s), 7.87 (1H, d, J=9.3 Hz), 8.03 (1H, d, J=9.3 Hz), 8.30 (1H, d, J=8.5 Hz), 9.30 (1H, d, J=8.1 Hz).

VIII: MP 215~217°C (dec), SI-MS m/z 494 $(M+Na)^+$, 472 $(M+H)^+$; UV λ_{max}^{MeOH} nm 220 (ε 35,200), 231 (37,400), 300 (4,520), 358 (4,660); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.16 (3H, d, J=6.8 Hz), 1.22~1.65 (5H, m), 2.09 (1H, br d, J=13.0 Hz), 2.63 (3H, s), 3.26 (1H, br d, J=12.0 Hz), 3.76 (1H, d, J=13.0 Hz), 4.09 (1H, d, J=15.6 Hz), 4.16 (1H, d, J=15.6 Hz), 4.37 (2H, m), 4.99 (1H, d, J=4.3 Hz), 7.53 (1H, d, J=8.6 Hz), 7.60 (2H, m), 7.83 (1H, s), 7.87 (1H, d, J=8.1 Hz), 7.95 (1H, d, J=9.4 Hz), 9.35 (1H, br).

IX: MP 180~183°C, SI-MS m/z 480 $(M+Na)^+$, 458 $(M+H)$. UV $\lambda_{max}^{H_2O}$ nm 219 (ε 31,200), 232 (35,000), 302 (5,400), 359 (4,580); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.17 (3H, d, J=6.8 Hz), 1.23~1.64 (4H, m), 1.72 (1H, br d, J=11.1 Hz), 2.29 (1H, br d, J=12.8 Hz), 3.33 (1H, br t, J=12.6 Hz), 3.53 (1H, m), 3.60 (1H, dd, J=17.1 and 5.8 Hz), 3.80 (1H, dd, J=17.1 and 5.8 Hz), 4.12 (1H, br d, J=12.6 Hz), 5.07 (1H,

d, J=3.0 Hz), 5.46 (1H, m), 7.65 (2H, m), 7.90 (1H, d, J=11.5 Hz), 7.91 (1H, s), 8.04 (1H, d, J=9.0 Hz), 8.17 (1H, t, J=5.8 Hz), 9.35 (1H, br).

X: MP 184~187°C, SI-MS m/z 591 $(M+Na)^+$, 569 $(M+H)^+$; UV λ_{max}^{MeOH} nm 220 (ε 38,900), 231 (40,500), 307 (6,700), 361 (5,000); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.14 (3H, d, J=7.0 Hz), 1.3~1.8 (10H, m), 1.91 (1H, m), 2.26 (1H, br d, J=11.0 Hz), 2.80 (1H, m), 3.10 (1H, br d, J=12.5 Hz), 3.26 (1H, br t, J=12.5 Hz), 3.46 (1H, d, J=11.5 Hz), 3.53 (1H, dd, J=17.2 and 4.8 Hz), 3.66 (1H, dd, J=17.2 and 4.8 Hz), 4.16 (1H, m), 4.26 (1H, br t, J=12.5 Hz), 5.08 (1H, d, J=3.3 Hz), 5.48 (1H, dd, J=6.2 and 2.9 Hz), 7.62 (2H, m), 7.87 (1H, s), 7.88 (1H, m), 7.89 (1H, m), 8.06 (1H, d, J=8.0 Hz), 8.26 (1H, d, J=8.1 Hz), 9.39 (1H, br).

XI: MP 179~182°C, SI-MS m/z 640 $(M+H)^+$; UV λ_{max}^{MeOH} nm 215 (35,700), 232 (36,600), 299 (5,500), 358 (4,860); ^{1}H NMR (400 MHz, D_2O+DCl) δ 1.23 (3H, d, J=7.0 Hz), 1.35~1.85 (9H, m), 1.91 (1H, br d, J=12.8 Hz), 2.14 (1H, br d, J=15.0 Hz), 2.30 (1H, br t, J=13.2 Hz), 2.80 (3H, s), 3.30 (1H, dt, J=12.0 and 2.9 Hz), 3.46 (1H, dt, J=13.0 and 2.2 Hz), 3.58 (1H, br d, J=12.0 Hz), 3.97 (1H, d, J=18.0 Hz), 4.03 (1H, d, J=18.0 Hz), 4.18 (1H, d, J=16.1 Hz), 4.23 (1H, d, J=16.1 Hz), 4.35 (1H, br d, J=13.2 Hz), 4.43 (1H, m), 5.11 (1H, dd, J=3.3 and 1.2 Hz), 5.22 (1H, dd, J=3.3 and 1.2 Hz), 5.71 (1H, d, J=3.7 Hz), 7.83 (1H, m), 7.92 (1H, m), 8.05 (1H, d, J=8.4 Hz), 8.19 (1H, d, J=8.4 Hz), 8.47 (1H, s).

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