A NEW PYRROLE-AMIDINE ANTIBIOTIC TAN-868 A

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A new pyrrole-amidine antibiotic TAN-868 A was isolated from the culture broth of *Streptomyces idiomorphus* sp. nov. Its chemical structure was determined by spectroscopic analyses and degradation studies to be 4-[(2*S*,4*R*)-4-hydroxy-5-iminoprolyl]amino-*N*-(2-amidinoethenyl)-2-pyrrolecarboxamide. The antibiotic is active against bacteria, fungi and a protozoan, and has cytotoxic activity against murine tumor cells. DNA thermal denaturation studies suggest that TAN-868 A preferentially interacts with AT rich regions of double-stranded DNA.

In our search for new antibacterial antibiotics, we detected strong activity against a multiple drug sensitive strain of *Micrococcus flavus* in a culture fluid of a streptomycete strain C-70895[1]. Two active metabolites isolated from the filtrate were designated TAN-868 A and B. Of these antibiotics, TAN-868 A (**1**) is a new oligopeptide antibiotic and TAN-868 B (**2**) was identical to kikumycin A[2,3] (Fig. 1). This paper describes the taxonomy of the producing organism, fermentation, isolation, chemical structures and biological activities of the antibiotics.

Materials and Methods

Taxonomic Studies

Culture characterization was carried out following the International Streptomyces Project procedure[4]. The color recorded for the mature culture was described according to the Color Harmony Manual[5]. Carbohydrate utilization was investigated by the method of PRIDHAM and GOTTLIEB[6]. Cell analysis was performed by the method of HASEGAWA *et al.*[7]. Scanning electron microscopy was conducted by the method of TANIDA *et al.*[8] with a Hitachi model S-570 scanning electron microscope.

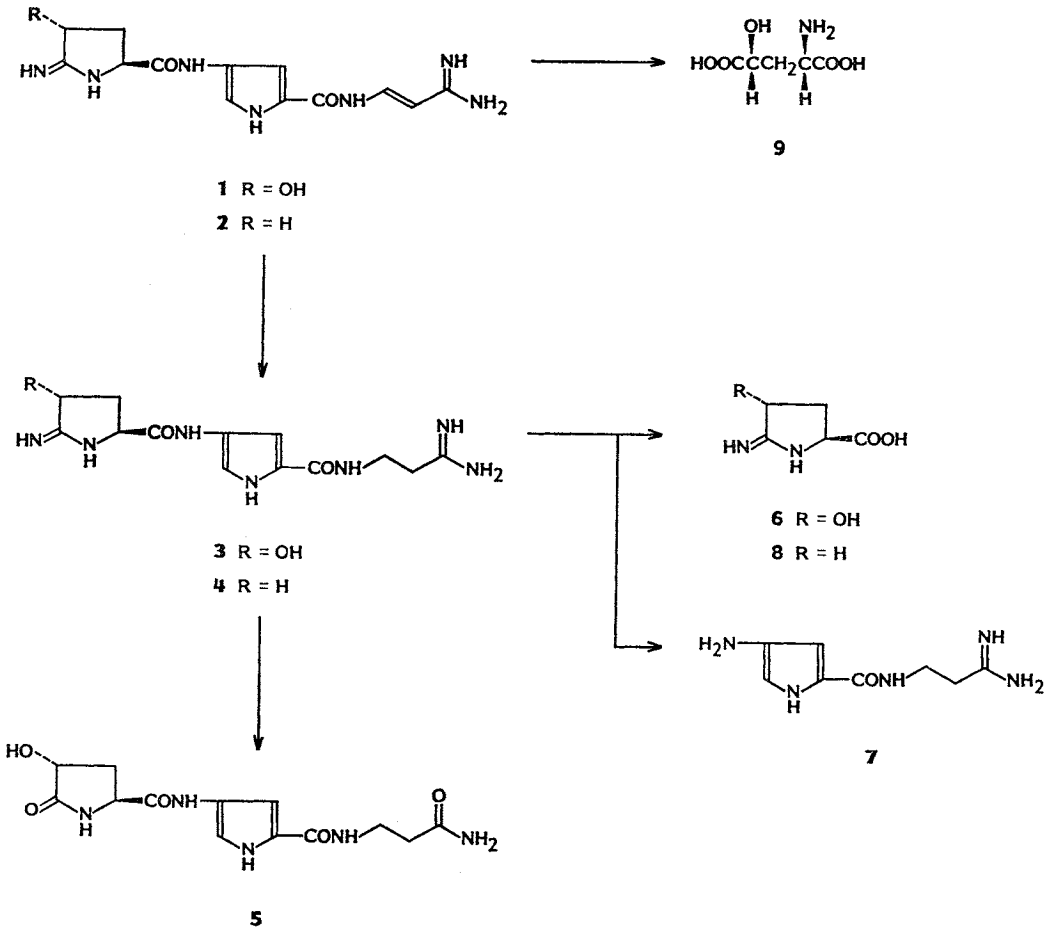
Assay for Antibiotic Production

The amount of the antibiotic in the fermentation broth was determined by paper-disk method using *Micrococcus flavus* IFO 3242 as the test organism and by reverse-phase high performance liquid chromatography (HPLC) using a ODS column of YMC-312 (Yamamura Chemical Lab.) with a mobile phase of acetonitrile - 0.01 M phosphate buffer (6 : 94).

Fermentation

A loopful of a strain C-70895 grown on mature slant culture was inoculated into a 2-liter flask containing 500 ml of a sterile seed medium. The flask was shaken on a reciprocal shaker at 28°C for 48 hours. The seed medium consisted of glucose 2%, cornsteep liquor 1%, soybean flour 1%, peptone 0.5%, NaCl 0.3% and $CaCO_3$ 0.5%. Five hundred milliliters of the seed culture was transferred to 30 liters of the same medium in a 50-liter fermentor. The fermentation was carried out at 28°C for 48 hours with aeration of 30 liters/minute and agitation of 280 rpm. Thirty liters of the seed culture was transferred to 1,200 liters of a production medium consisting of dextrin 5%, cornsteep liquor 3%, peptone 0.1%, $CaCl_2$ 1% and $CaCO_3$ 0.5%, in a 2,000-liter fermentor. The fermentation was carried out at 30°C with aeration of 1,200 liters/minute and agitation of 150 rpm.

Fig. 1. Degradation pathways of TAN-868 A (1) and B (2).



Measurement

The specific rotations and UV spectra were measured in H_2O at 22~23°C. The IR spectra were measured in KBr pellets. The δ values were recorded in ppm downfield from TMS in the ^1H NMR (90 MHz) spectra using a Varian EM-390 spectrometer. The proton spin-decoupling studies of **1** were carried out by using a Jeol GX-400 (400 MHz) in D_2O. The secondary ionization mass spectra (SI-MS) were measured on a Hitachi M-80 A mass spectrometer with a xenon ion beam source. The samples were supplied in a glycerol matrix.

Isolation of **1** and **2**

The culture broth (100 liters) adjusted to pH 6 to 7 was filtered using Hyflo-Super Cel. The filtrate (98 liters, at pH 6~7) was applied to a chromatography column containing Amberlite IRC-50 (H^+ type, 5 liters). After washing the column with H_2O, the active metabolites were eluted with 0.2 N HCl (25 liters). The active fractions were applied to a column of Diaion HP-10 (<50 mesh, 5 liters) and eluted with MeOH - 0.01 N HCl (1 : 1, 25 liters). The concentrate of the eluate (4.8 liters, at pH 6) was chromatographed on Amberlite CG-50 (H^+ type, 400 ml) and eluted with 0.1 N HCl (400 ml). After neutralization, the solution was loaded on a column of Diaion SP-207 (200 ml) and eluted with MeOH - 0.01 N HCl (1 : 1, 900 ml). The active fractions were concentrated and freeze-dried to afford a crude powder. The crude powder was purified by chromatography using CM-Sephadex C-25 (H^+ type, 200 ml) and eluted with 0.2 N NaCl (2.5 liters). Desalination of the eluates was carried out by column chromatography using activated carbon (100 ml) followed by elu-

tion with 8% 2-BuOH. The concentrate of the eluates thus obtained (3 lots) was applied to a column of Diaion HP-20 (100~200 mesh, 200 ml) and eluted with H_2O. The fractions corresponding to single peaks by HPLC were combined, concentrated and freeze-dried to give **1** (665 mg) and **2** (150 mg) as white powders of the dihydrochloride salts.

When an aqueous solution of the dihydrochloride of **1** (100 mg) was adjusted to pH 3.0~3.5 by 0.5 N H_2SO_4, TAN-868 A sulfate was obtained as colorless crystals (97 mg).

TAN-868 A·H_2SO_4: MP >300°C (dec); $[\alpha]_D$ +53.3° (*c* 1.0, 1 N HCl); UV λ_{max} nm (ε) 231 (17,400), 320 (34,400); IR ν_{max} cm^{-1} 1665, 1585, 1480, 1415, 1375.

Anal Calcd for $C_{13}H_{17}N_7O_3 \cdot H_2SO_4 \cdot H_2O$: C 35.86, H 4.86, N 22.52, S 7.36.
Found: C 35.57, H 4.81, N 22.38, S 7.55.

Hydrogenation of **1** and **2** (**3** and **4**)

The dihydrochloride of **1** (200 mg) in H_2O (50 ml) was hydrogenated over Pd-black (50 mg) at room temp. The catalyst was filtered off and the filtrate was freeze-dried to give a white powder of **3** (200 mg).

$[\alpha]_D$ +53.1° (*c* 0.55); UV λ_{max} nm (ε) 234 (14,900), 276 (13,200); IR ν_{max} cm^{-1} 1700, 1660, 1595, 1550; SI-MS *m/z* 322 $(M+H)^+$; ^1H NMR δ 2.75 (2H, m), 2.97 (2H, t, *J*=6 Hz), 3.93 (2H, t, *J*=6 Hz), 5.00 (1H, m), 5.43 (1H, t, *J*=7 Hz), 7.07 (1H, d, *J*=2 Hz), 7.47 (1H, d, *J*=2 Hz).

Anal Calcd for $C_{13}H_{19}N_7O_3 \cdot 2HCl \cdot \frac{1}{2}H_2O$: C 38.72, H 5.50, N 24.31, Cl 17.58.
Found: C 38.38, H 5.50, N 23.99, Cl 17.35.

Compound **2** (300 mg) was hydrogenated by the same way described above to afford a compound **4** (295 mg) as a white powder.

$[\alpha]_D$ +17.4° (*c* 0.50); UV λ_{max} nm (ε) 233 (15,300), 276 (13,500); IR ν_{max} cm^{-1} 3270, 1700, 1585, 1535; SI-MS *m/z* 306 $(M+H)^+$; ^1H NMR δ 2.52 (1H, m), 2.87 (1H, m), 2.97 (2H, t, *J*=6 Hz), 3.23 (2H, t, *J*=7 Hz), 3.93 (2H, t, *J*=6 Hz), 5.00 (1H, m), 7.07 (1H, d, *J*=1 Hz), 7.47 (1H, d, *J*=1 Hz).

Anal Calcd for $C_{13}H_{19}N_7O_2 \cdot 2HCl \cdot 2H_2O$: C 37.69, H 6.08, N 23.67, Cl 17.11.
Found: C 37.69, H 6.00, N 23.29, Cl 17.25.

Alkaline Hydrolysis of **3** (**5**)

A solution of **3** (55 mg) in 0.1 M Na_2HPO_4 was adjusted to pH 9.7 with 1 N NaOH. The solution was stirred for 6.5 hours at 60°C. The reaction mixture was adjusted to pH 6 and chromatographed on Diaion HP-20 (50~100 mesh, 10 ml) followed by elution with 10% MeOH. The eluate was concentrated and freeze-dried to afford a white powder of **4** (32 mg). An analytical sample was obtained by crystallization from H_2O.

MP 233~236°C (dec); $[\alpha]_D$ +50.1° (*c* 0.49); UV λ_{max} nm (ε) 231 (14,400), 275 (12,500); IR ν_{max} cm^{-1} 3320, 1720, 1660, 1600, 1560; electron impact mass spectra (EI-MS) *m/z* 323 (M^+), 252, 196, 125; ^1H NMR δ 2.67 (2H, m), 2.77 (2H, t, *J*=6 Hz), 3.80 (2H, t, *J*=6 Hz), 4.5~5.0 (2H, m), 7.00 (1H, d, *J*=2 Hz), 7.43 (1H, d, *J*=2 Hz).

Anal Calcd for $C_{13}H_{17}N_5O_5$: C 48.30, H 5.30, N 21.66.
Found: C 48.01, H 5.57, N 21.51.

Acid Hydrolysis of **3** (**6** and **7**)

A solution of **3** (300 mg) in 2 N HCl (30 ml) was refluxed for 1 hour. The mixture was evaporated to dryness. The residue was dissolved in H_2O (50 ml) and the solution was applied to a column of Dowex 50W-X2 (50~100 mesh, H^+ type, 15 ml). The column was washed with H_2O and eluted with 0.5 N HCl and 2 N HCl. The eluate of 0.5 N HCl was evaporated to dryness and the residue was dissolved in H_2O. After adjustment to pH 6.8, the solution was desalted with activated carbon (20 ml). The eluate was concentrated and lyophilized. The resulting powder was treated with MeOH - Et_2O to give colorless crystals of **6** (75 mg).

MP 198~202°C; $[\alpha]_D$ +55.5° (*c* 0.65); IR ν_{max} cm^{-1} 3050, 1730, 1590; SI-MS *m/z* 145 $(M+H)^+$; ^1H NMR δ 2.67 (2H, m), 4.67 (1H, dd, *J*=3.9 Hz), 5.28 (1H, t, *J*=8 Hz).

Anal Calcd for $C_5H_8N_2O_3$: C 41.67, H 5.59, N 19.44.
Found: C 41.48, H 5.68, N 19.21.

The eluate of 2 N HCl was evaporated to dryness. The residue was dissolved in H_2O, freeze-dried

and precipitated with addition of acetone to give a white powder of **7** (197 mg).

IR ν_{max} cm^{-1} 3360, 3090, 1695, 1635, 1585, 1550, 1540; SI-MS *m/z* 196 $(M+H)^+$; ^1H NMR δ 2.98 (2H, t, *J*=7 Hz), 3.95 (2H, t, *J*=7 Hz), 7.10 (1H, d, *J*=2 Hz), 7.43 (1H, d, *J*=2 Hz).

Anal Calcd for $C_8H_{13}N_5O \cdot 2HCl \cdot 2H_2O$: C 31.59, H 6.30, N 23.02.
Found: C 31.64, H 6.13, N 23.31.

Compounds **7** (185 mg) and **8** (78 mg) were obtained from **4** (270 mg) in the same way described above.

7: The physico-chemical data were identical with those of the compound obtained from **3**.

8: MP 229~232°C (dec); $[\alpha]_D$ −16.9° (*c* 0.48); IR ν_{max} cm^{-1} 3100, 1700, 1600; SI-MS *m/z* 129 $(M+H)^+$; ^1H NMR δ 2.42 (1H, m), 2.80 (1H, m), 3.12 (2H, t, *J*=7 Hz), 4.65 (1H, dd, *J*=6.9 Hz).

Anal Calcd for $C_5H_8N_2O_2 \cdot \frac{1}{2}H_2O$: C 43.79, H 6.61, N 20.43.
Found: C 43.37, H 6.67, N 19.95.

Acid Hydrolysis of **1** (**9**)

A solution of **1** (500 mg) in 6 N HCl (25 ml) was refluxed for 24 hours. The mixture was evaporated to dryness. The residue was dissolved in H_2O (100 ml) and adjusted to pH 7.4. The solution was applied to a column of Dowex 1-X2 (AcO^- type, 50~100 mesh, 40 ml). The column was washed with H_2O and 0.2 N CH_3COOH successively, and active fractions were eluted with 0.5 N CH_3COOH. The eluate was evaporated to dryness and the residue was dissolved in H_2O. The solution was decolorized with charcoal and freeze-dried. The resulting powder was treated with EtOH - H_2O to give colorless crystals of **9** (80 mg).

MP 124~126°C (dec); $[\alpha]_D$ +17.7 (*c* 0.51); IR ν_{max} cm^{-1} 3475, 3170, 1735, 1635; SI-MS *m/z* 164 $(M+H)^+$; ^1H NMR δ 2.1~2.9 (2H, m), 4.22 (1H, dd, *J*=6 and 8 Hz), 4.67 (1H, dd, *J*=3 and 9 Hz).

Anal Calcd for $C_5H_9NO_5 \cdot H_2O$: C 33.15, H 6.12, N 7.73.
Found: C 33.18, H 5.88, N 7.86.

Assay for Antimicrobial Activity

The activity against bacteria, yeasts and fungi was determined by an agar dilution method. Trypticase soy agar (BBL) was used as the assay medium for common bacteria. The medium was supplemented with glycerol 3% for acid-fast bacteria and glucose 1% for yeasts and fungi, respectively. The activity against *Tetrahymena pyriformis* W was assayed by a dilution method[9]. Bacteria were grown for 18 hours at 37°C, whereas yeasts, fungi and the protozoan were grown for 48 hours at 28°C.

Assay for Cytotoxic Activity

Murine tumor cell lines, P-815 mastocytoma, EL-4 and YAC-1 lymphoma were purchased from The American Type Culture Collection. B-16 melanoma was obtained from The Japanese Cancer Research Resources Bank. Mouse fibroblast L-929 was maintained in our Research Division. The cytotoxic activities were evaluated by the MTT assay[10]. RPMI 1640 with 10% fetal calf serum was used for YAC-1 cells. RPMI 1640 with 10% horse serum was used for EL-4 cells. Eagle MEM with 10% fetal calf serum was used for L-929, P-815 and B-16 cells. The cells of 5×10^4/ml were incubated with an antibiotic at 37°C for 72 hours in an atmosphere of 5% CO_2 and 95% air.

Thermal Denaturation Study

Thermal denaturation studies of double-stranded DNAs were carried out using a Gilford model 250 spectrophotometer attatched to a model 2527 thermoprogramer. A heating rate of 0.5°C/minute was employed.

Chemicals

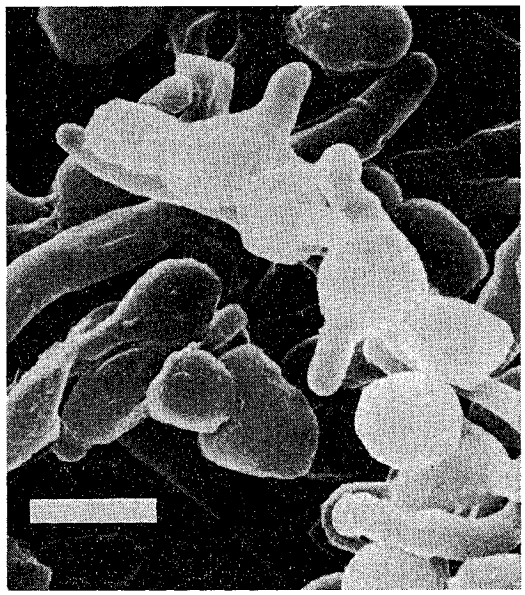
Distamycin A was obtained from Sigma Chemical Co. (U.S.A.). Polydeoxyribonucleotides were purchased from Pharmacia P-L Biochemicals, Inc. (U.S.A.). RPMI 1640, Eagle MEM and serums were obtained from M. A. Bioproducts, Inc. (U.S.A.).

Results and Discussion

Taxonomy of the Producing Organism

Strain C-70895 was isolated from a soil sample collected in Formosa. Microscopic and scanning electron microscopic studies of the organism show that straight and flexuous aerial mycelia are formed from branched mycelia grown in both nutritionally rich and chemically defined agar media. Spore chains are hooks, open loops, or greatly extended coils of large diameter, and consist of about 20 spores per chain. The spores are spherical, oval, or cylindrical ($0.6\sim1.0\times0.6\sim0.9$ μm). The spore surface is smooth, and forms a lateral bud (Plate 1). The cultural and physiological characteristics of strain C-70895 are shown in Table 1. The color of mature sporulated aerial mycelium is in the Yellow or Gray series. The strain does not produce melanoid pigment. Cell analysis of the strain showed the presence of LL-diaminopimelic acid, and it was classified as chemotype I/NC. These characteristics of strain C-70895 indicate that it belongs to the genus *Streptomyces*. However there is no related species in BERGEY's Manual of Determinative Bacteriology, 8th Ed.[11] nor in The Actinomycetes[12]. The formation of lateral buds, has been observed so far only in the genus *Pseudonocardia*[13]. For this reason it was named *Streptomyces idiomorphus* sp. nov. C-70895. The organism is deposited at the Institute for Fermentation, Osaka, under the accession number IFO 14492. Strain C-70895 differs from *Streptomyces phaeochromogenes* R-719, the producer of kikumycins A and B[2], in its aerial mass color, lack of melanin production, and its lateral bud formation.

Plate 1. Scanning electron micrograph of the spores of strain C-70895 on yeast extract - malt extract agar after 2 weeks of cultivation at 28°C (bar; 1 μm).



Production and Isolation

A typical time course for the fermentation is shown in Fig. 2. Antibiotic **1** was produced beginning at about 30 hours and reached a maximum at about 42 hours. Fig. 3 shows the isolation procedure of **1** and **2**. These basic, water-soluble antibiotics were purified with column chromatography using cation-exchange resins and Sephadex and followed by desalination using activated carbon and adsorptive resins. In a final stage, two active fractions were separated by fine Diaion HP-20 chromatography. The fractions including another compound were successively separated into two components each by rechromatography on preparative reverse-phase HPLC. Antibiotics **1** and **2** were obtained as freeze-dried dihydrochlorides of white powders and **1** as monosulfate of colorless crystals. They show positive color reactions to Greig-Leaback, *p*-dimethylaminobenzaldehyde, potassium permanganate and Ehrlich (acidic) reagents, and negative color reactions to ninhydrin, Sakaguchi, Ehrlich (basic) and Dragendorff reagents. They are easily soluble in water, soluble

Table 1. Cultural and physiological characteristics of strain C-70895.

Cultural characteristics	
Yeast extract - malt extract agar (ISP-2)	G[a] : Moderate A : Moderate, powdery; light ivory (2ca) to natural (2dc) R : Amber (3lc) to russet orange (4nc) S : Pale yellow (1ca)
Oatmeal agar (ISP-3)	G : Moderate A : Moderate; ivory tint (2cb) to natural (2dc) R : Cream (1 1/2ca) S : None
Inorganic salts - starch agar (ISP-4)	G : Moderate A : Poor; white to ivory tint (2cb) R : Colorless S : None
Glycerol - asparagine agar (ISP-5)	G : Moderate A : Moderate, powdery; light ivory (2ca) to light wheat (2ea) R : Colonial yellow (2ga) to amber (3nc) S : None
Sucrose - nitrate agar (Waksman 1)	G : Moderate A : Poor, powdery, light ivory (2ca) R : Colorless to yellow maple (3ng) S : None
Glucose - asparagine agar (Waksman 2)	G : Poor A : None R : Colorless S : None
Physiological characteristics	
Temperature range for growth	16~35°C (optimum 27~34°C)
Nitrate reduction	Positive
Starch hydrolysis	Negative
Milk peptonization	Positive
Gelatin liquefaction	Positive
Melanoid pigment production	Negative
Utilization of carbon sources	Positive: D-Glucose, *i*-inositol Negative: L-Arabinose, D-mannitol, D-xylose, D-fructose, sucrose rhamnose, raffinose, cellulose

[a] G: Growth, A: aerial mass color, R: reverse side color, S: soluble pigment.

in dimethyl sulfoxide or methanol and sparingly soluble in ethyl acetate or diethyl ether.

The physico-chemical and spectral data of **1** and **2** are summarized in Table 2. The molecular formulae of **1** and **2** were determined on the basis of the elemental analyses, the molecular ion peaks in SI-MS and carbon numbers in ^{13}C NMR spectrometry as shown in Table 3. These correspond to $C_{13}H_{17}N_7O_3(2HCl)$ and $C_{13}H_{17}N_7O_2(2HCl)$, respectively. The UV spectra of these antibiotics showed two maxima at 230 and 321 nm. These data indicate that TAN-868 components have a chromophore similar to pyrrole-amidine antibiotics, kikumycins[2,3]. Compound **2** was identical with kikumycin A and **1** was different from **2** in the specific rotation, molecular formula and NMR spectra.

Structural Elucidation

When compared with the ^{13}C NMR spectra of **1** and **2** (Table 3), either methylene signal at 32.49 or 28.62 ppm in **2** was observed as a methine signal in **1** at 72.99 ppm (d). This evidence is consistent

Fig. 2. Time course of active metabolites production by strain C-70895 in a 2,000-liter fermentor.
● Total antibiotics, ○ growth, --- pH.

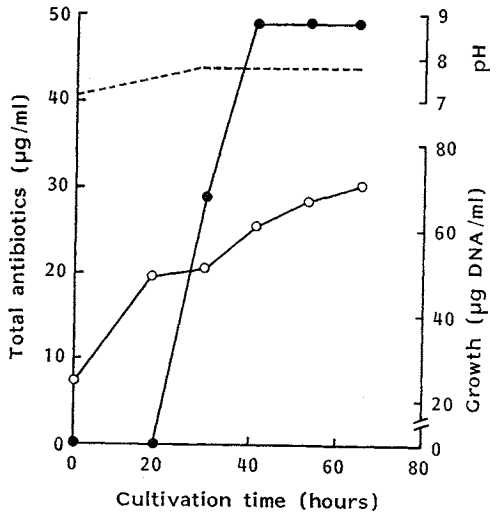
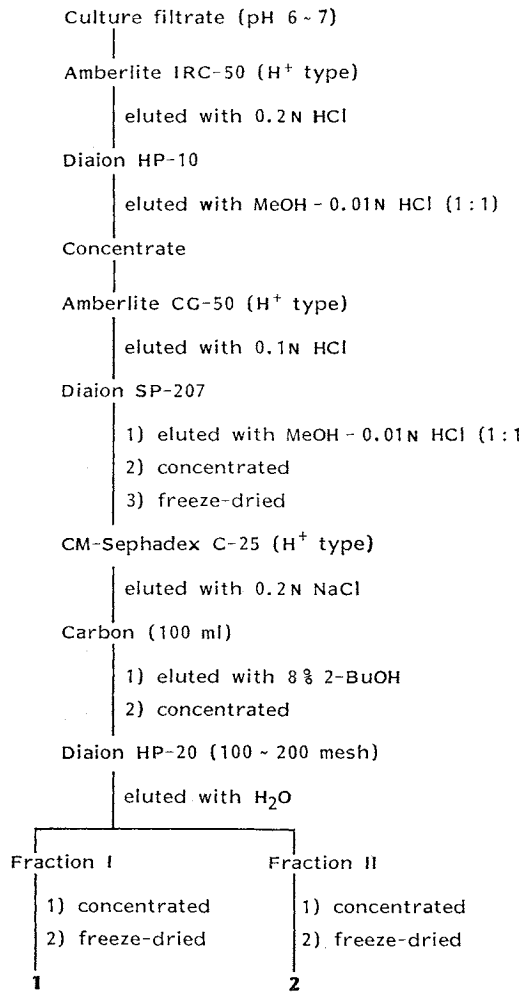


Fig. 3. Isolation procedure of **1** and **2** (hydrochloride).



with the presence of a secondary hydroxyl group in **1**. The ^{1}H NMR spectrum of **1** also indicated the presence of a freshly generated methine signal at 5.23 ppm instead of the methylene signal at 3.20 ppm in **2** (Table 3). The relations of protons in the proline moiety were confirmed by the proton spin-decoupling studies as follows: On irradiation of the methine signal at 4.80 ppm, two proton signals of the methylene group at 2.73 ppm and 2.51 ppm were collapsed. When the methylene signals were independently irradiated, the methine signal at 5.23 ppm was decoupled in both cases.

Fig. 1 shows the degradation pathways of **1**. Compounds **1** and **2** gave a dihydro derivatives (**3** and **4**, respectively) by hydrogenation using Pd-black in water. On mild alkaline hydrolysis **3** afforded a dideamino compound (**5**) whose EI-MS showed the corresponding fragmentation patterns at *m/z* 323 (M^+), 252, 196 and 125 as indicated by TAKAISHI *et al.*[14]. On acidic hydrolysis by refluxing in 2 N HCl for 1 hour, **3** and **4** afforded proline analogues (**6** and **8**, respectively) and an aminopyrrole compound (**7**, commonly). These degradation data confirmed the sequence of three moieties and the chemical structure of **1**.

The absolute configurations of **1** were established as follows: Hydrolysis of **1** by refluxing in 6 N HCl gave 4-hydroxyglutamic acid (**9**) originating from the proline moiety. The molar rotations of **9**, $[M]_D$ +32.1° (*c* 0.51, H_2O) or +62.8° (*c* 0.53, 5 N HCl), were in good accord to those of [2*S*,4*R*]-4-hydroxyglutamic acid, $[M]_D$ +31.8° (*c* 1, H_2O) or +61.6° (*c* 1, 5 N HCl)[15]. Thus the structure of **1** was finally determined to be 4-[(2*S*,4*R*)-4-hydroxy-5-iminoprolyl]amino-*N*-(2-amidinoethenyl)-2-

Table 2. Physico-chemical properties of TAN-868 A (**1**) and B (**2**) (hydrochloride).

Property	**1**	**2**
Appearance	White powder	White powder
$[\alpha]_D$ (*c*)[a]	+61.6° (1.05)	+19.3° (1.1)
SI-MS $(M+H)^+$	320	304
Molecular formula	$C_{13}H_{17}N_7O_3 \cdot 2HCl(2H_2O)$	$C_{13}H_{17}N_7O_2 \cdot 2HCl(1\frac{1}{2}H_2O)$
Anal	Found Calcd	Found Calcd
	C 36.24, 36.46	C 38.30, 38.72
	H 5.30, 5.41	H 5.53, 5.50
	N 22.90, 22.89	N 24.23, 24.31
	Cl 16.96, 16.56	Cl 17.34, 17.58
	O 18.68	O 13.89
UV λ_{max} nm (ε)[a]	230 (17,200), 321 (33,300)	230 (15,900), 321 (31,400)
IR ν_{max} (KBr) cm^{-1}	1670, 1580, 1470, 1370, 1290, 1225, 1090, 950	1660, 1580, 1470, 1370, 1280, 1220, 1080, 950
TLC[b] Rf (1)	0.55	0.53
(2)	0.16	0.17
HPLC[c] Rt (minutes)	4.7	5.2

[a] The data were measured at 23~25°C in water.

[b] Adsorbent: Spot film, Cellulose f (Tokyo kasei); solvent system, (1) PrOH - pyridine - AcOH - water (15 : 10 : 3 : 12), (2) BuOH - AcOH - water (4 : 1 : 1); detection, UV lamp at 254 nm.

[c] Equipment: Liquid chromatograph 638-50 (Hitachi): column, ODS, YMC-Pack A-312 (Yamamura Chem. Lab.); mobile phase, 27% acetonitrile - 0.01 M sodium octane sulfonate, 0.02 M phosphoric acid (pH 3.0); detection, UV absorbance at 214 and 254 nm; flow rate, 2 ml/minute. Rt: Retention time.

Table 3. NMR spectral data of TAN-868 A (**1**) and B (**2**) in D_2O.

Assignment	**1**		**2**	
	^{13}C (100 MHz) ppm	^1H (400 MHz) ppm (*J*, Hz)	^{13}C (100 MHz) ppm	^1H (90 MHz) ppm (*J*, Hz)
CO	174.81 (s)		175.60 (s)	
	171.91 (s)		172.88 (s)	
C(=NH)N	166.56 (s)		166.64 (s)	
	162.16 (s)		162.28 (s)	
C=	125.48 (s)		125.46 (s)	
	124.40 (s)		124.45 (s)	
CH=	140.52 (d)	8.16 (d, *J*=14.7)	140.60 (d)	8.13 (d, *J*=15)
	120.21 (d)	5.87 (d, *J*=14.7)	120.35 (d)	5.93 (d, *J*=15)
	109.20 (d)	7.40 (d, *J*=1.7)	109.41 (d)	7.53 (d, *J*=2)
	100.11 (d)	7.11 (d, *J*=1.7)	100.06 (d)	7.21 (d, *J*=2)
CHOH	72.99 (d)	5.23 (t, *J*=8.3)	32.49 (t)[a]	3.20 (2H, m)
CH(NH–)CO	62.00 (d)	4.80 (dd, *J*=2.2, 9.0)	64.59 (d)	4.85 (m)
CH_2	38.05 (t)	2.73 (ddd, *J*=2.2, 8.0, 13.5),	28.62 (t)[a]	2.87 (m)
		2.51 (dt, *J*=8.7, 9.0, 13.5)		2.47 (m)

[a] These methylene signals may be reversed.

pyrrolecarboxamide. Similarly hydrolysis of **2** (kikumycin A) by refluxing in 6 N HCl gave glutamic acid, whose absolute configuration was determined to be the L-form by the HPLC method described in the literature[16]. The absolute configuration of **2** was also confirmed as shown in Fig. 1.

Biological Activities

Antibiotic **1** showed a broad antibacterial activity, and was especially active against *Micrococcus flavus* IFO 3242, *Micrococcus luteus* IFO 12708, *Bacillus subtilis* PCI 219, *Bacillus megaterium* IFO 12108, *Mycobacterium phlei* IFO 3158 and *Acinetobacter calcoaceticus* IFO 13006 as shown in Table 4. It was more active than **2** and distamycin A against bacteria. This antibiotic showed protective effects in mice experimentally infected with *Escherichia coli* O-111 and *Staphylococcus aureus* 308 A-1 by subcutaneous administration as shown in Table 5 (M. KONDO, unpublished data). The preliminary acute toxicities (LD_{50}) of **1** in mice are 25~100 mg/kg by intraperitoneal injection, *ca.* 50 mg/kg by subcutaneous injection and >400 mg/kg by oral administration (S. CHIBA, personal communication). The cytotoxic activity of **1** is shown in Table 6. **1** was active against murine tumor cells. **2** was active not only against tumor cells but also against L-929 fibroblasts. **1** did not show antitumor activity against P388 leukemia *in vivo*.

Pyrrole-amidine antibiotics, such as netropsin and distamycin A, are known to interact with AT rich regions of double-stranded DNA and they inhibit the growth of microorganisms and animal cells by inhibiting DNA-dependent DNA and RNA polymerase systems[17,18]. To clarify whether **1** interacts with DNA, thermal denaturation studies were carried out (Fig. 4). **1** raised the melting temperatures of poly(dA-dT)poly(dA-dT) and poly(dA)poly(dT). However, **1** scarcely shifted the melting temperature of poly(dG-dC)poly(dG-dC). Distamycin A and **2** also showed similar profiles. These

Table 4. Antimicrobial activity of TAN-868 A (**1**), kikumycin A (**2**) and distamycin A.

Test organism	MIC (μg/ml)		
	TAN-868 A	Kikumycin A	Distamycin A
Escherichia coli K-12	50	50	>100
E. coli NIHJ JC2	50	50	>100
Proteus mirabilis ATCC 21100	20	100	>100
P. vulgaris IFO 3045	20	100	>100
Klebsiella pneumoniae IFO 3317	>100	>100	>100
Serratia marcescens IFO 3046	50	50	>100
Salmonella typhimurium IFO 12529	>100	>100	>100
Citrobacter freundii IFO 12681	50	100	>100
Pseudomonas aeruginosa IFO 3080	>100	>100	>100
Alcaligenes faecalis IFO 13111	20	50	>100
Acinetobacter calcoaceticus IFO 13006	5	10	20
Bacillus subtilis PCI 219	10	10	50
B. cereus IFO 3514	100	100	20
B. megaterium IFO 12108	10	20	20
Staphylococcus aureus FDA 209P	20	20	50
Micrococcus luteus IFO 12708	5	20	20
M. flavus IFO 3242	1	1	20
Mycobacterium smegmatis ATCC 607	>100	>100	100
M. phlei IFO 3158	10	50	100
M. avium IFO 3154	>100	>100	20
Candida albicans IFO 0583	100	100	>100
Cryptococcus neoformans IFO 0410	50	50	50
Rhodotorula rubra IFO 0907	>100	>100	>100
Saccharomyces cerevisiae IFO 0209	20	20	50
Aspergillus niger IFO 4066	>100	50	>100
Penicillium chrysogenum IFO 4626	100	50	100
Tetrahymena pyriformis W	100	>100	100

Table 5. Protective effects of TAN-868 A (1) in experimentally infected mice (ICR).

Organism	Antibiotic	MIC (μg/ml)[a]	ED_{50} (mg/kg)[b]
Staphylococcus aureus 308 A-1	TAN-868 A	12.5	4.82
	Cefmenoxime	1.56	6.25
Escherichia coli O-111	TAN-868 A	12.5	4.05
	Cefmenoxime	0.025	0.032

[a] Inoculum size; 10^8 cfu/ml, medium; Trypticase soy agar.
[b] Infection; ip challenge (10^8 cfu/mouse), therapy; sc administration immediately after the challenge.

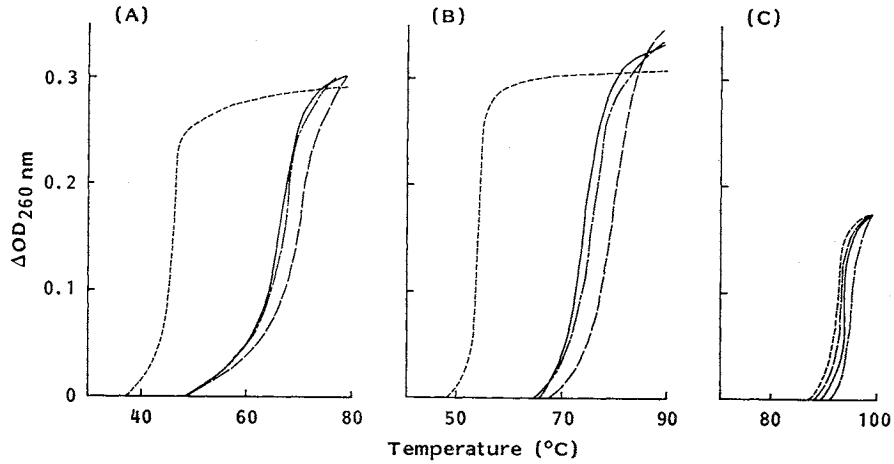
Table 6. Cytotoxic activity of TAN-868 A (1), kikumycin A (2) and distamycin A.

Cell lines	IC_{50} (μg/ml)		
	TAN-868 A	Kikumycin A	Distamycin A
L-929 (fibroblast)	>100	82	>100
B-16 (melanoma)	40	48	17
P-815 (mastocytoma)	22	22	13
EL-4 (lymphoma)	21	9.7	4.4
YAC-1 (lymphoma)	20	11	9.5

Fig. 4. Effect of TAN-868 A (1), kikumycin A (2) and distamycin A on thermal denaturation of various DNAs.

A concentration of each DNA was prepared at an OD_{260} of 0.5. An antibiotic at 10 μM was added to each DNA solution: Drug free control (------), TAN-868 A (——), kikumycin A (-·-) and distamycin A (---).

(A) Poly(dA-dT)poly(dA-dT) in SSC (SSC=0.15 M NaCl plus 0.015 M sodium citrate); (B) poly-(dA)poly(dT) in SSC; (C) poly(dG-dC)poly(dG-dC) in 0.02×SSC.



results suggest that 1 interacts with AT rich regions of double-stranded DNA. The growth inhibiting activity of 1 against microorganisms and murine tumor cells may be explained by inhibition of DNA and RNA syntheses in the target cells.

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