ISOLATION, CHARACTERIZATION AND STRUCTURES OF PA-46101 A AND B

MAKOTO MATSUMOTO, YOSHIMI KAWAMURA, YOHKO YOSHIMURA, YOSHIHIRO TERUI, HIROSHI NAKAI, TADASHI YOSHIDA and JUN'ICHI SHOJI

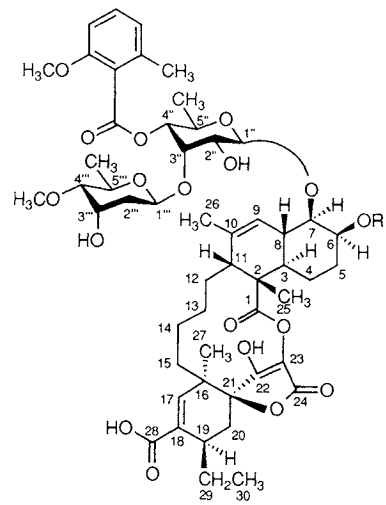
Shionogi Research Laboratories, Shionogi & Co., Ltd.,
5-12-4 Fukushima-ku, Sagisu, Osaka 553, Japan

(Received for publication October 27, 1989)

New antibiotics, PA-46101 A and B, were isolated from the culture broth of a Streptomycete. The molecular formulae of A and B were determined to be $C_{52}H_{70}O_{18}$ and $C_{61}H_{86}O_{22}$, respectively, by elemental analyses, NMR and mass spectrometry. Their structures were elucidated by X-ray crystallography and NMR spectroscopy. These antibiotics are active *in vitro* against anaerobic Gram-positive and Gram-negative bacteria and also against a limited number of aerobic Gram-positive bacteria.

In a screening program for new antibiotics with dominant activity against anaerobic bacteria, we found PA-46101 A (**1**) and B (**2**) as metabolites of *Streptomyces* strain PA-46101 (Fig. 1). The structure of antibiotic PA-46101 A was elucidated by X-ray crystallography and shown to contain a tetronic acid moiety and a macrocyclic ring such as in kijanimicin[1], chlorothricin[2] and tetrocarcin[3,4]. In this paper, the taxonomy of the producing strain, the production and isolation of the antibiotics as well as their physico-chemical and biological properties are presented.

Fig. 1. Structures of PA-46101 A (**1**) and B (**2**)



PA-46101 A (**1**) R=H
PA-46101 B (**2**) R=2,4-Di-*O*-methyl-3-*C*-methyl-α-(D or L)-rhamnosyl

Taxonomy of the Producing Strain

Morphology

The vegetative mycelia of the strain PA-46101 grow well on both synthetic and organic media, and do not show fragmentation into coccoid or bacillary elements. Abundant aerial mycelia are formed on inorganic salts-starch agar. The spore chains are of the *Spira* type and have less than twenty spores per chain. The spores are cylindrical in shape, and have smooth surface. Sporangia, screrotia and flagellated spores were not observed.

Chemical Composition

LL-2,4-Diaminopimelic acid (DAP) was detected in the whole-cell hydrolysate of the strain PA-46101 by the method of BECKER *et al.*[5].

Cultural and Physiological Characteristics

Cultures were observed after incubation at 28°C for 2 weeks. The cultural and physiological

Table 1. Cultural characteristics of strain PA-46101.

Media	Growth	Sporulation	Color of aerial mycelium	Reverse color	Soluble pigment
Sucrose - nitrate agar	Moderate	Poor	Brownish white	Pale yellowish brown	None
Glucose - asparagine agar	Poor	None	—	Pale yellowish brown	None
Glycerol - asparagine agar	Moderate	Moderate	Brownish white	Pale brown	Pale brown
Inorganic salts - starch agar	Good	Good	Pale orange	Pale yellowish brown	None
Tyrosine agar	Good	Poor	Brownish white	Pale yellowish brown	None
Nutrient agar	Good	Poor	Brownich white	Pale yellowish brown	Pale yellowish brown
Yeast extract - malt extract agar	Good	Poor	Brownish white	Light brown	Light brown
Oatmeal agar	Good	Poor	Brownish white	Pale brown	None
BENNETT's agar	Good	Moderate	Brownish white	Brown	Brown (trace)

characteristics of strain PA-46101 are summarized in Tables 1 and 2, respectively. Color names described in Table 1 are designated on the basis of the color table in "Guide to Color Standard", published by Nippon Shikisai Kenkyusho, Tokyo, Japan.

Table 2. Physiological properties of strain PA-46101.

Production of melanoid pigment	Negative
Tyrosinase reaction	Negative
Coagulation of milk (28°C)	Positive
Peptonization of milk (28°C)	Positive
Hydrolysis of starch	Positive
Liquefaction of gelatin (28°C)	Positive

Carbon Utilization

The utilization of carbon sources was studied on PRIDHAM and GOTTLIEB's basal agar containing 1% of each carbon sources at 28°C. D-Glucose, D-xylose, L-arabinose, D-fructose, L-fructose, L-rhamnose, raffinose and D-mannitol were utilized; sucrose and inositol were not utilized.

The strain PA-46101 exhibits the following properties: Spore chain, *Spira*; spores, cylindrical and smooth surface; color of vegetative mycelia, pale yellowish brown to brown; color of aerial mycelia, brownish white to pale orange; soluble pigment, none to pale yellowish brown; DAP in whole-cell hydrolysate, LL-type.

Based on the taxonomic properties described above, the strain PA-46101 is considered to belong to the genus *Streptomyces*.

Fermentation

A 3-liter Erlenmeyer flask containing 800 ml of a medium consisting of soluble starch 0.5%, glucose 0.5%, Polypepton 0.5%, beef extract 0.5%, yeast extract 0.25% and NaCl 0.25% (pH 7.0) was inoculated with the spores of strain PA-46101. The broth was cultured at 28°C for 2 days on a rotatory shaker at 180 rpm. Eight hundred ml of the culture was transferred to a 30-liter jar fermenter containing 20 liters of a medium consisting of dextrin 2%, tomato paste 2% and dry yeast 1% (pH 7.0). Fermentation was carried out at 28°C for 4 days under aeration of 20 liters per minute and agitation of 350 rpm.

Isolation and Purification

The fermented broth (74 liters) was adjusted with dil HCl to pH 3.0 and centrifuged. The mycelial cake was extracted with 80% aqueous acetone (18 liters), and the extract was concentrated *in vacuo* to

about 5 liters. The concentrate was extracted with ethyl acetate (5 liters) at pH 3.0. The broth supernatant was also extracted with ethyl acetate (21 liters). Both ethyl acetate extracts were combined and concentrated to 12 liters. The antibiotic was transferred to water at pH 9.0 and re-extracted with ethyl acetate (18 liters) at pH 3.0. The extract was washed with water and concentrated to about 1 liter. On addition of petroleum ether, the antibiotic complex was precipitated to give a red powder (5.07 g).

The crude powder was chromatographed on a silica gel column (Merck, 2×95 cm) with $CHCl_3$-MeOH (98:2). Active eluate fractions were evaporated *in vacuo*. The residue was applied to a column of Diaion CHP-20P (Mitsubishi Chemical Industries, 2×32 cm) and eluted with 20 mM phosphate buffer (pH 7.5) and the same buffer containing 60% acetonitrile in the form of a linear gradient. Active fractions eluted in 40~55% acetonitrile were concentrated *in vacuo* and adjusted to pH 3.0 with dil HCl, and the compounds were extracted with ethyl acetate. The extract was concentrated to a small volume and chromatographed on a silica gel column (1.5×19 cm) with $CHCl_3$-MeOH (98:2). The active eluate was concentrated to give PA-46101 A as colorless crystals (359 mg). The mother liquor was chromatographed on a column of LiChroprep RP-18 (Merck, 2.5×31 cm) and eluted with a mixture of 20 mM phosphate buffer, pH 7.5, and acetonitrile (30:70). Small amounts of PA-46101 A were eluted in the effluent volume 100~130 ml and B in volume 150~170 ml. The latter fraction was concentrated and extracted with ethyl acetate at pH 3.0. The extract was further purified by chromatography on a silica gel column (5×15 mm) with CH_2Cl_2-MeOH (98:2) to give PA-46101 B as a colorless powder (29 mg).

Physico-chemical Properties

PA-46101 A and B are acidic substances. These antibiotics are soluble in methanol and chloroform, slightly soluble in ethyl acetate and benzene, but insoluble in hexane and water. They show positive reactions with Dragendorff reagent and sulfuric acid, but a negative reaction with ferric chloride on silica gel plates. The IR spectra of PA-46101 A and B show nearly identical absorptions at 1770, 1710, 1670

Fig. 2. IR Spectra of PA-46101 A (**1**) and B (**2**) (KBr).

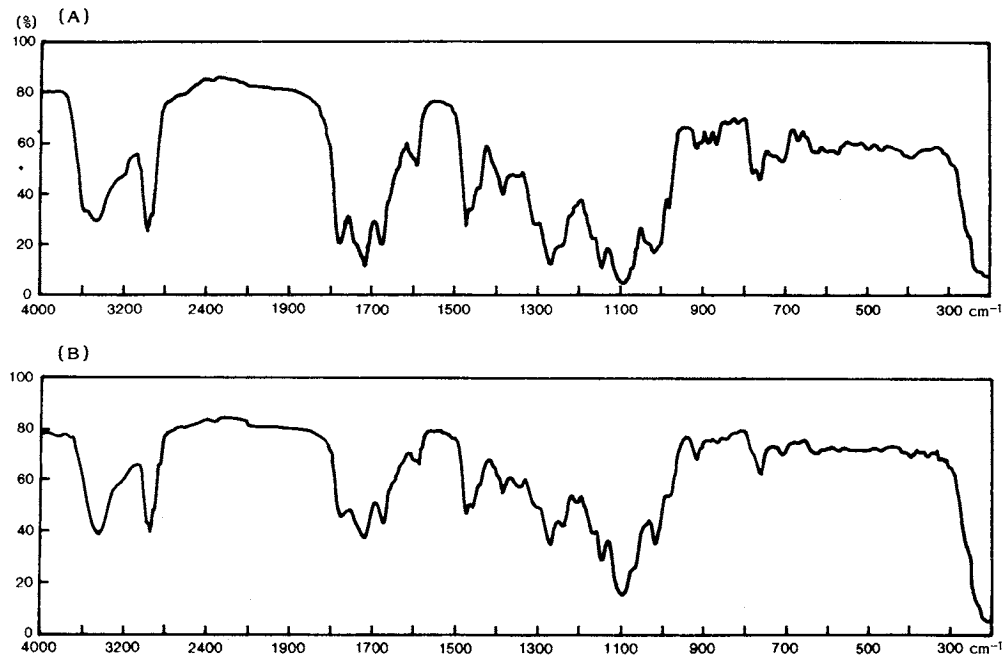


Fig. 3. UV Spectra of PA-46101 A (**1**) and B (**2**).

—— MeOH, —·— 0.1 N HCl in MeOH, --- 0.1 N NaOH in MeOH.

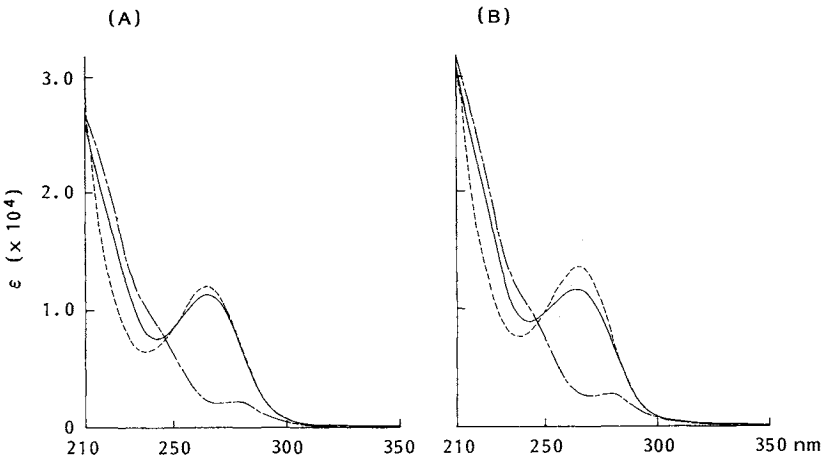


Table 3. Physico-chemical properties of PA-46101 A and B.

	PA-46101 A	PA-46101 B
Appearance	Colorless crystal	Colorless powder
MP (°C)	263~266	225~227
Anal	$C_{52}H_{70}O_{18}\cdot H_2O$	$C_{61}H_{86}O_{22}\cdot 4H_2O$
	Calcd: C 62.38, H 7.25	Calcd: C 58.93, H 7.62
	Found: C 62.81, H 7.65	Found: C 59.14, H 6.94
SI-MS (*m/z*, $(M+H)^+$)	983	1,171
UV λ_{max}^{MeOH} nm (ε)	265 (12,500)	264 (11,600)
$\lambda_{max}^{0.1\,N\,HCl\text{-}MeOH}$ nm (ε)	280 (2,500)	280 (2,900)
$\lambda_{max}^{0.1\,N\,NaOH\text{-}MeOH}$ nm (ε)	265 (13,300)	265 (13,500)
IR (KBr) cm^{-1}	3440, 1770, 1710, 1670, 1585, 1263, 1138, 1083, 1008, 757	3440, 1772, 1713, 1672, 1585, 1264, 1142, 1090, 1010, 759
$[\alpha]_D^{24}$	+26° (*c* 0.50, MeOH)	−0.9° (*c* 0.457, MeOH)
TLC (Rf)[a]	0.37	0.40
HPLC (Rt)[b]	5.2	8.0

[a] Merck Silica gel 60 ($CHCl_3$-MeOH, 9:1).

[b] Develosil$_7$ C_{18} 4.6×250 mm: CH_3CN-20 mM phosphate buffer, pH 7.5 (35:65).

and 1585 cm^{-1}, indicating the presence of a tetronic acid, an ester and a lactone groups (Fig. 2). As shown in Fig. 3, their UV spectra show an absorption maximum at 264~265 nm in methanol, which shifted to 280 nm in acid. These data and some other physico-chemical properties are summarized in Table 3. Their ^1H and ^{13}C NMR data are listed in Tables 4 and 5, respectively. These signals are assigned by ^1H-^1H COSY, relayed COSY, rotating-frame nuclear Overhauser enhancement spectroscopy (ROESY), ^{13}C-^1H COSY and long-range ^{13}C-^1H COSY spectra of PA-46101 A.

Structure Elucidation

The structure of PA-46101 A (**1**) was determined by X-ray crystallographic analysis. The perspective view of the molecular structure is shown in Fig. 4. The absolute configuration of PA-46101 A was established as follows. The acid methanolysis afforded two methyl glycosides, α-anomer (**3**) ($[\alpha]_D^{24}$ +10.6° (*c* 0.26, CH_3OH)) and β-anomer (**4**) ($[\alpha]_D^{24}$ +185.7° (*c* 0.1, CH_3OH)). The ^1H NMR data of **3** and **4** were in good

Table 4. ^{1}H NMR data[a] of PA-46101 A (**1**) and B (**2**) in $CDCl_3$ at 400 MHz.

Proton	1	2	Proton	1	2
Aglycon			Sugar		
3-H	1.45 m[b]	1.4 m[b]	1′-H	—	5.30 br s
4-H$_\alpha$	1.55 m[b]	[b]	2′-H	—	3.27 d (J=1.2)
4-H$_\beta$	1.48 m[b]	[b]	4′-H	—	2.92 d (J=9.7)
5-H$_\alpha$	1.55 m[b]	1.50 m[b]	5′-H	—	3.65 dq (J=9.7, 6.2)
5-H$_\beta$	2.08 br d (J=10)	1.88 m[b]	6′-H	—	1.27 d (J=6.2)
6-H	4.19 m	4.20 m[b]	2′-OCH$_3$	—	3.54 s
7-H	3.29 dd (J=10.8, 2.7)	3.19 dd (J=10.9, 2.3)	3′-CH$_3$	—	1.34 s
8-H	2.33 br t (J=10)	2.30 br t (J=10)	4′-OCH$_3$	—	3.59 s
9-H	5.75 br s	5.82 br s	1″-H	4.62 d (J=8.0)	4.57 d (J=8.2)
11-H	1.83 br d (J=6)	1.83 br d (J=6)	2″-H	3.56 dd (J=8.0, 2.8)	3.41[b]
12-H	0.90 m[b,c]	0.90 m[b,c]	3″-H	4.43 t (J=2.8)	4.39 t (J=2.8)
	1.55 m[b,c]	1.55 m[b,c]	4″-H	4.78 dd (J=10.1, 2.8)	4.66 dd (J=10.0, 2.8)
13-H	0.66 br q (J=11)[c]	0.66 br q (J=11)[c]	5″-H	4.00 dq (J=10.1, 6.1)	3.96 dq (J=10.0, 6.1)
	1.83 m[b,c]	[b,c]	6″-H	1.28 d (J=6.1)	1.27 d (J=6.1)
14-H	0.96 m[b]	[b]	Benzoyl		
	1.50 m[b]	[b]	3-H	6.76 dd (J=8.5, 0.9)	6.76 d (J=8.3)
15-H	1.33 m[b]	[b]	4-H	7.25 t (J=8.0)	7.26 t (J=7.9)
	1.45 m[b]	[b]	5-H	6.80 dd (J=7.5, 0.9)	6.81 dd, (J=7.6, 0.8)
17-H	6.55 d (J=1.5)	6.56 d (J=1.6)	2-OCH$_3$	3.83 s	3.83 s
19-H	2.58 br t (J=8)	2.59 br t (J=9)	6-CH$_3$	2.31 s	2.30 s
20-H$_\alpha$	2.46 dd (J=14.5, 7.5)	2.46 dd (J=15.0, 8.3)	Sugar		
20-H$_\beta$	1.88 br d (J=14.5)	1.90 br d (J=15.0)	1‴-H	4.85 dd (J=9.2, 2.1)	4.83 dd (J=9.5, 2.3)
25-H	1.36 s	1.32 s	2‴-H$_\alpha$	2.16 br d (J=13)	2.16 br d (J=13)
26-H	1.71 t (J=1.7)	1.69 t (J=1.5)	2‴-H$_\beta$	1.6 m[b]	[b]
27-H	1.24 s	1.24 s	3‴-H	4.21 q (J=3.0)	4.20 q (J=3.0)
29-H	1.71 m[b]	[b]	4‴-H	2.86 dd (J=9.8, 3.0)	2.87 dd (J=9.5, 3.0)
	1.75 m[b]	[b]	5‴-H	3.83 dq (J=9.8, 6.4)	3.84 dq (J=9.5, 6.2)
30-H	0.92 t (J=7.4)	0.93 t (J=7.3)	6‴-H	1.31 d (J=6.4)	1.30 d (J=6.2)
28-COOH	10.24 br s	10.22 br s	4‴-OCH$_3$	3.41 s	3.41 s

[a] ^{1}H Chemical shifts (δ_H), signal multiplicities and coupling constants (J in Hz) in parentheses.
[b] These signals overlap with other signals.
[c] Assignments may be reversed.

agreement with those reported for methyl 2,6-dideoxy-4-*O*-methyl-α-L-*ribo*-hexopyranoside (**5**) ($[\alpha]_D^{26}$ −12.4° (CH_3OH)) and β-anomer (**6**) ($[\alpha]_D^{26}$ −209.2° (CH_3OH))[1], respectivery. Since the latter compounds **5** and **6** derived from kijanimicin are of the L-configuration, the sugar of PA-46101 A was established to be of the D-configuration. The opposite signs in $[\alpha]_D$ values of **3** and **4** indicated that **3** is 2,6-dideoxy-4-*O*-methyl-α-D-*ribo*-hexopyranose and that **4** is the β-anomer. Thus, the structure and the absolute configuration of PA-46101 A was determined as shown in Fig. 1.

The SI-MS, and ^{1}H and ^{13}C NMR data suggest that PA-46101 B (**2**) has an additional sugar moiety attached to the molecule of A. The structure of B and its sugar moiety (**7**) was clarified by analyses of NOE's, chemical shifts and coupling constants as follows. A methyl doublet observed at δ 1.27 ($J_{5'ax,6'}$=6.2 Hz, 6-CH_3) indicates that the sugar is a 6-deoxy-sugar. The large coupling constant of $J_{4',5'}$=9.7 Hz between 4′-H at δ 2.92 and 5′-H at δ 3.65 shows that 4′-H and 5′-H are *trans*-diaxial. While an NOE could not be observed between 4′-H and 3′-CH_3, its absence suggests that 4′-H and 3′-CH_3 are in *trans*-diaxial relationship. The NOE between 2′-H at δ 3.27 and 3′-CH_3 at δ 1.34 was observed, showing that 2′-H and 3′-CH_3 are *cis* oriented. The chemical shift of the anomeric proton 1′-H at δ 5.30 and the small J value ($J_{1'eq,2'eq}$=1.2 Hz) indicates that both the protons are in equatorial position[6]. Thus, the

Table 5. ^{13}C Chemical shifts of PA-46101 A (**1**) and B (**2**) in $CDCl_3$ at 100 MHz.

Carbon	1	2	Carbon	1	2
Aglycon			C-3′	—	73.16
C-1	179.85	179.83	C-4′	—	86.62
C-2	50.35	50.42	C-5′	—	66.72
C-3	37.65	37.90	C-6′	—	18.02
C-4	19.98	20.71	2′-OCH_3	—	59.02
C-5	29.90	31.04	3′-CH_3	—	18.53
C-6	67.62	77.03	4′-OCH_3	—	61.64
C-7	85.12	85.34	C-1″	101.91	103.51
C-8	36.68	37.43	C-2″	70.23	70.92
C-9	119.73	120.03	C-3″	80.21	81.06
C-10	134.51	134.34	C-4″	74.01	74.33
C-11	54.15	54.19	C-5″	67.48	67.34
C-12	30.37[a]	30.53[a]	C-6″	17.60	17.82
C-13	33.69[a]	33.73[a]	Benzoyl		
C-14	25.74	25.79	COO	167.73	167.82
C-15	34.34	34.41	C-1	123.09	123.21
C-16	46.47	46.54	C-2	156.30	156.35
C-17	148.08	148.20	C-3	108.23	108.31
C-18	131.23	131.24	C-4	130.43	130.48
C-19	34.03	34.12	C-5	122.26	122.33
C-20	27.61	27.65	C-6	136.09	136.05
C-21	82.88	82.92	2-OCH_3	55.77	55.86
C-22	160.91	160.92	6-CH_3	19.15	19.18
C-23	116.33	116.44	Sugar		
C-24	165.87	165.88	C-1‴	99.40	99.69
C-25	15.86	15.86	C-2‴	36.26	36.29
C-26	22.41	22.38	C-3‴	63.62	63.72
C-27	21.62	21.70	C-4‴	82.11	82.10
C-28	171.59	171.47	C-5‴	68.68	68.75
C-29	26.44	26.51	C-6‴	17.93	18.02
C-30	12.59	12.65	4‴-OCH_3	57.35	57.41
Sugar					
C-1′	—	98.14			
C-2′	—	84.92			

[a] Assignments may be reversed.

Fig. 4. Perspective view of PA-46101 A (**1**) with the atom-numbering system.

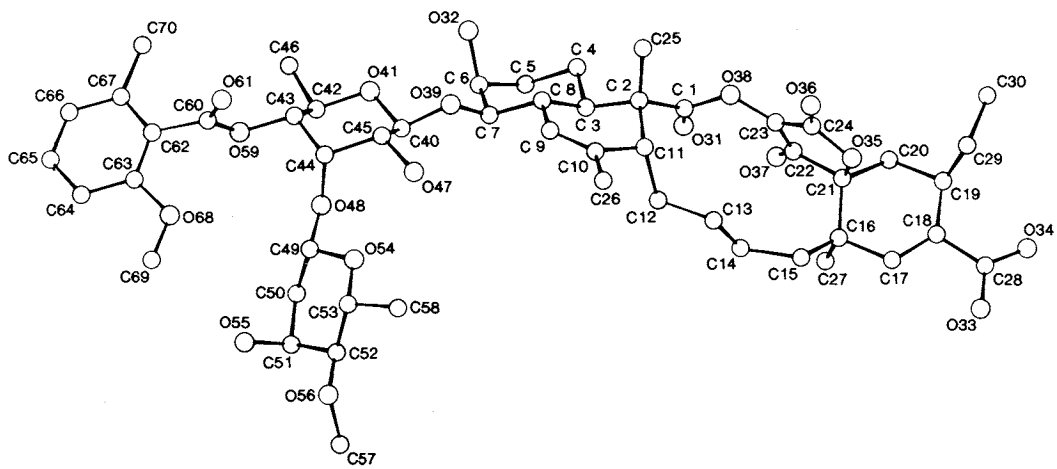
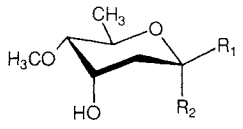
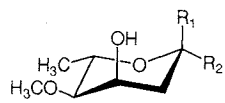
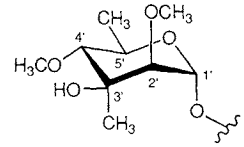


Fig. 5. Sugar components of PA-46101 A (**1**) and B (**2**).







3 $R_1=H$ $R_2=OCH_3$
4 $R_1=OCH_3$ $R_2=H$

5 $R_1=OCH_3$ $R_2=H$
6 $R_1=H$ $R_2=OCH_3$

7

Table 6. Antibacterial spectra of PA-46101 A and B against anaerobic bacteria.

Test Organism	MIC (μg/ml)	
	A	B
Peptococcus asaccharolyticus ATCC 14963	0.05	0.05
P. prevotii ATCC 9321	—	12.5
P. micros VPI 5464-1	—	3.13
Streptococcus constellatus ATCC 27823	—	0.2
Eubacterium limosum ATCC 8486	12.5	0.39
E. aerofaciens ATCC 25986	—	>100
Propionibacterium acnes ATCC 11827	3.13	0.39
Bifidobacterium adolescentis JCM 1250	—	6.25
B. bifidum JCM 1122	1.56	3.13
B. longum ATCC 15707	6.25	3.13
Clostridium perfringens ATCC 13124	1.56	6.25
C. difficile ATCC 17857	12.5	0.39
Veillonella parvula ATCC 10790	1.56	>100
Bacteroides fragilis GM 7000	>100	100
B. fragilis ATCC 25285	—	>100
B. thetaiotaomicron WAL 3304	6.25	>100
B. vulgatus ATCC 29327	>100	12.5
B. melaninogenicus GAI 0413	>100	>100
Fusobacterium varium ATCC 8501	100	>100
F. necrophorum ATCC 25286	>100	>100
F. nucleatum ATCC 25586	25	50
F. mortiferum ATCC 9817	>100	>100

Inoculum size: One loopful of 10^6 cfu/ml.
Medium: GAM Agar (Nissui).

structure of the sugar moiety is assumed to be 2,4-di-*O*-methyl-3-*C*-methyl-D-rhamnose (**7**) or its enantiomer, 2,4-di-*O*-methyl-3-*C*-methyl-L-rhamnose, as shown in Fig. 5. This structure was supported by the evidence that the ^{13}C chemical shift of 3'-CH_3 (δ 18.53) is similar to that of sugars which have the same partial structure of C-1' to C-5'[7]. By comparison of the ^{13}C chemical shift of C-6 carbon (δ 77.03) of B with that (δ 67.62) of A, the glycosidation shift ($\Delta\delta+9.41$) was observed. Thus, it was shown that the sugar is linked through its anomeric oxygen atom to the C-6 position via an α-linkage. Therefore, the structure of PA-46101 B (**2**) was deduced as shown in Fig. 1.

Biological Properties

PA-46101 A and B show strong activity against anaerobic Gram-positive bacteria and show weak activity against anaeroic Gram-negative bacteria and a few species of aerobic Gram-positive bacteria (Tables 6 and 7). They do not show antitumor and antifungal activity.

Table 7. Antibacterial spectra of PA-46101 A and B against aerobic bacteria.

Test organism	MIC (μg/ml)	
	A	B
Staphylococcus aureus FDA JC-1	>100	>100
S. aureus Smith	>100	>100
Streptococcus pyogenes C-203	0.78	0.78
S. pneumoniae Type 1	1.56	3.13
Escherichia coli NIHJ JC-2	>100	>100
E. coli EC-14	>100	>100
Klebsiella pneumoniae SR1	>100	>100
Proteus vulgaris CN-329	>100	>100
Enterobacter cloacae ATCC 13047	>100	>100
Serratia marcescens ATCC 13880	>100	>100
Pseudomonas aeruginosa ATCC 25619	>100	>100

Experimental

The UV absorption spectra were measured with a Hitachi 323 spectrometer, IR absorption spectra with a Jasco DS-403G spectrometer, $[\alpha]_D$ with a Perkin-Elmer 241 polarimeter, SI-MS with a Hitachi M-90 mass spectrometer and ^1H and ^{13}C NMR spectra with a Varian XL-400 (^1H 399.94 MHz, ^{13}C 100.577 MHz) or a VXR-200 (^1H 200.057 MHz) spectrometer in $CDCl_3$ with tetramethylsilane as an internal standard.

X-Ray Crystallographic Analysis of PA-46101 A (1)

The molecular structure of PA-46101 A was determined by X-ray analysis. Colorless prismatic crystals grown in an ethyl acetate solution containing a small amount of methanol were suitable for the measurement.

Crystal data: Monoclinic; space group C2; $a=34.380$ (4), $b=10.406$ (1), $c=23.889$ (3) Å, $\beta=114.32$ (1)°; $Z=4$. Intensity data were collected by $\omega-2\theta$ scan on a Rigaku diffractometer with graphite-monochromatized Cu $K\alpha$ radiation. A crystal ($0.2\times0.4\times0.7$ mm) was enclosed in a thin glass-capillary with mother liquid. Intensities were measured in the range $\theta\leqq55°$ with a variable scan range $(1.1+0.2\tan\theta)°$ and a constant scan speed of 5° minute^{-1}. 5,174 independent intensities were corrected for Lorentz and polarization factors, but not for absorption effects.

The structure was solved by MULTAN84[8]. A perspective view of the molecule, along with the atom-numbering system, is shown in Fig. 4[9]. Hydrogen atoms, except for the one bonded to O (55), were located on the difference density map calculated after block-diagonal least-squares refinement. The crystal structure had a large vacant space between the molecules, but no significant peaks for solvent molecules were found in the map, except for one peak which was considered to be a fragment of the solvent molecules. Successive refinement of the positional parameters and anisotropic thermal parameters of the non-hydrogen atoms gave an R value ($\Sigma|\Delta F|/\Sigma|F_o|$) of 0.070 for 2,856 reflections ($F_o>3\sigma$) in the range of $8.9<\theta\leqq55°$. In the refinements the temperature factor of each hydrogen atom was set equal to B_{eq} of the bonded atom, and the H atoms of the hydrocarbons were fixed at their ideal positions. In addition, the occupancy of the fragment of the solvent molecules was estimated to be about 0.15 from the refinements using an isotropic temperature factor. The weighting scheme used was $w=[\sigma^2(F_o)+0.00221|F_o|^2]^{-1}$ for $w^{1/2}|F_c|\geqq3$ and $w^{1/2}|\Delta F|<3$, $w=0$ otherwise.

Significant peaks were not found in the final difference density map. It thus appears that the solvent molecules are probably disordered in the space. The molecule forms a dimer structure around a 2-fold axis with intermolecular hydrogen bonds between carboxyl groups, O (33) H–O (34) (2.64 (1) Å). Other OH groups form intramolecular hydrogen bonds of O (32) H–O (39) (2.73 (1) Å), O (37) H–O (31) (2.65 (1)), O (47) H–O (54) (2.73 (1)) and probably O (55) H–O (56) (2.74 (2)).

Methanolysis of PA-46101 A

PA-46101 A (50 mg) was dissolved in 1.8 N HCl-MeOH (3 ml) and the solution was allowed to stand

at 25°C for 18 hours. The solution was neutralized with $NaHCO_3$, filtered, and the filtrate was evaporated to dryness *in vacuo*. The residue was chromatographed on a silica gel column (1 × 4 cm) with CH_2Cl_2 - MeOH (98 : 2). Concentration of the eluate gave a residue (10.6 mg). The residue was further purified by preparative TLC on silica gel plates (Merck, Silica gel 60 F_{254}) with CH_2Cl_2 - EtOAc (2 : 8). The bands corresponding to **3** (Rf 0.42) and **4** (Rf 0.58), detected by coloration with H_2SO_4, were extracted with CH_2Cl_2 - MeOH (95 : 5) and evaporated *in vacuo* to give **3** (3.2 mg) and **4** (1.4 mg), respectively. Compound **3** was obtained as a colorless oil; $[\alpha]_D^{24}$ +10.6° (*c* 0.26, CH_3OH) (literature[1]), $[\alpha]_D^{26}$ −12.4° (CH_3OH) for **5**); EI-MS *m/z* 333 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$) δ 1.30 (3H, d, J=6.2 Hz, 6-CH_3), 1.63 (1H, ddd, J=2.9, 9.5 and 13.9 Hz, 2-H_{ax}), 2.18 (1H, ddd, J=2.1, 3.5 and 13.9 Hz, 2-H_{eq}), 2.87 (1H, dd, J=2.9 and 9.2 Hz, 4-H_{ax}), 3.42 (3H, s, 4-OCH_3), 3.47 (3H, s, 1-OCH_3), 3.77 (1H, dq, J=6.2 and 9.2 Hz, 5-H_{ax}), 4.26 (1H, ddd, J=2.9, 2.9 and 3.5 Hz, 3-H_{eq}), 4.72 (1H, dd, J=2.1 and 9.5 Hz, 1-H). Compound **4** was also obtained as a colorless oil; $[\alpha]_D^{24}$ +185.7° (*c* 0.1, CH_3OH) (literature[1]), $[\alpha]_D^{26}$ −209.2° (CH_3OH) for **6**); ^1H NMR ($CDCl_3$) δ 1.30 (3H, d, J=6.2 Hz, 6-CH_3), 1.86 (1H, ddd, J=3.5, 3.6 and 14.8 Hz, 2-H_{ax}), 2.17 (1H, ddd, J=1.4, 3.4 and 14.8 Hz, 2-H_{eq}), 2.84 (1H, dd, J=2.8 and 9.6 Hz, 4-H_{ax}), 3.32 (3H, s, 4-OCH_3), 3.38 (3H, s, 1-OCH_3), 3.95 (1H, dq, J=6.2 and 9.6 Hz, 5-H_{ax}), 4.21 (1H, ddd, J=2.8, 3.4 and 3.6 Hz, 3-H_{eq}), 4.75 (1H, dd, J=1.4 and 3.5 Hz, 1-H).

Acknowledgments

The authors are indebted to several colleagues of their laboratories for the elemental analyses, MS, UV and IR spectra.

References

1) MALLAMS, A. K.; M. S. PUAR & R. R. ROSSMAN: Kijanimicin. Part 3. Structure and absolute stereochemistry of kijanimicin. J. Chem. Soc. Perkin Trans. I 1983: 1497~1534, 1983
2) MUNTWYLER, R. & W. KELLER-SCHIERLEIN: The structure of chrolothricins, new macrolide antibiotics. Helv. Chim. Acta 55: 2071~2094, 1972
3) KASAI, M.; M. YOSHIDA, N. NIRAYAMA, F. TOMITA, T. TAMAOKI, K. SHIRAHATA, Y. SASADA & Y. OHASHI: Structure of new antitumor antibiotics, tetrocarcins. Abstracts Papers of the 23rd Symposium on the Chemistry of Natural Products, No. 76, p. 584, Nagoya, Oct. 22~25, 1980
4) TAMAOKI, T.; M. KASAI, K. SHIRAHATA, S. OHKUBO, M. MORIMOTO, K. MINEURA, S. ISHII & F. TOMITA: Tetrocarcins, novel antitumor antibiotics. II. Isolation, characterization and antitumor activity. J. Antibiotics 33: 946~950, 1980
5) BECKER, B.; M. P. LECHEVALIER, R. E. GORDON & H. A. LECHEVALIER: Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole-cell hydrolysates. Appl. Microbiol. 12: 421~423, 1964
6) HONG, N.; M. FUNABASHI & J. YOSHIMURA: Synthesis of L-nogalose and its enantiomer. Carbohydr. Res. 96: 21~28, 1981
7) SATO, K.; M. MATSUZAWA, K. AJISAKA & J. YOSHIMURA: Branched-chain sugars. XIX. On the application of ^{13}C NMR spectroscopy to the configurational assignment of 3-*C*-substituents of aldohexopyranose derivatives. Bull. Chem. Soc. Jpn. 53: 189~191, 1980
8) MAIN, P.; G. GERMAIN & M. M. WOOLFSON: MULTAN84. A Computer Program for the Automatic Solution of Crystal Structures from X-ray Diffraction Data. University of York (England) and Louvain (Belgium), 1984
9) MOTHERWELL, W. D. S. & W. CLEGG: PLUTO. Program for Plotting Molecular and Crystal Structures. Cambridge Crystallographic Data Centre, University Chemical Laboratory, 1978