COMMUNICATIONS TO THE EDITOR

Epoxyquinomicins A and B, New Antibiotics from *Amycolatopsis*

Sir:

In the course of a screening program for new antibiotics, we isolated new antibiotics, epoxyquinomicins A (**1**) and B (**2**) from the culture broth of *Amycolatopsis* sp. MK299-95F4, which was isolated from a soil sample collected at Sendai city, Miyagi Prefecture, Japan. In this paper we report the production, isolation, physico-chemical properties, biological properties and structures of **1** and **2** (Fig. 1).

A slant culture of the epoxyquinomicins-producing organism was inoculated into a 500 ml Erlenmeyer flask containing 110 ml of a seed medium consisting of glycerol 0.5%, sucrose 2%, soybean meal 1%, dry yeast 1%, corn steep liquor 0.5% and $CoCl_2$ 0.001% (adjusted to pH 7.0 before sterilization). The inoculated medium was incubated at 30°C for 5 days on a rotary shaker. Two ml of the seed culture was transferred to each 500 ml Erlenmeyer flask containing 110 ml of a producing medium. The producing medium was composed of glycerol 2.0%, dextrin 2.0%, Bacto-Soytone (Difco) 1.0%, yeast extract 0.3%, $(NH_4)_2SO_4$ 0.2% and $CaCO_3$ 0.2% (adjusted to pH 7.4 before sterilization). The fermentation was carried out at 27°C for 4 days on a rotary shaker.

The culture broth was filtrated and the filtrate (2.55 liters) was extracted with *n*-BuOAc at pH 2. The organic layer was concentrated and dried under reduced pressure. The crude material was dissolved in MeOH (50 ml) and the solution was washed twice with *n*-hexane (50 ml), followed by concentrating under reduced pressure to dryness. The dried residue was distributed in a mixture of $CHCl_3$ - MeOH - H_2O (100 ml, 50 : 10 : 40, v/v). Then the lower layer was concentrated *in vacuo* to yield a brown oil (0.515 g). The oily material was chromatographed on a silica gel column (Merck,

Fig. 1. The structures of epoxyquinomicins A (**1**) and B (**2**).

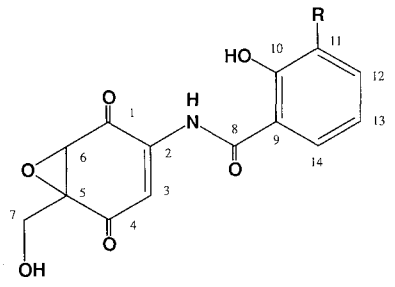


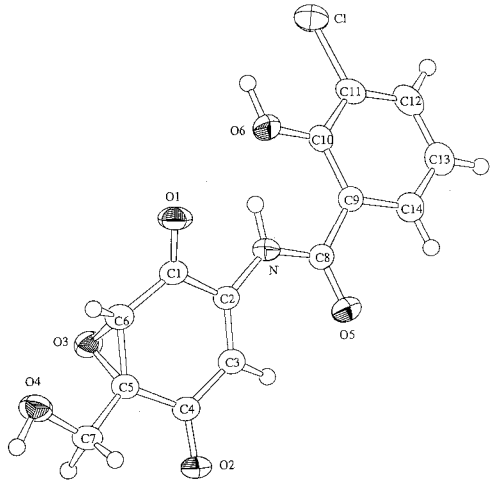
Table 1. Physico-chemical properties of epoxyquinomicins A (**1**) and B (**2**).

	1	2
Appearance	Pale yellow powder	Pale yellow powder
Nature	Acidic	Acidic
Molecular formula	$C_{14}H_{10}NO_6Cl$	$C_{14}H_{11}NO_6$
FAB-MS (*m/z*)	$(M+H)^+$ 324, 326	M^+ 289
	$(M-H)^-$ 322, 324	$(M-H)^-$ 288
HRFAB-MS (*m/z*)	Calcd: 322.0118	Calcd: 290.0664
	(as $C_{14}H_9NO_6Cl$)	(as $C_{14}H_{12}NO_6$)
	Found: 322.0136 $(M-H)^-$	Found: 290.0656$(M+H)^+$
UV λ max(ε)		
in MeOH	236 (sh, 8900), 255 (sh, 5900), 325 (8000), 370 (sh, 2700)	237(6100), 253 (sh, 5400), 326 (6300)
in 0.01N NaOH-MeOH	234 (sh, 11200), 257 (sh, 5100), 327 (8300), 371 (sh,4400)	235 (9100), 259 (sh, 4000), 324 (5800), 376 (sh, 3400)
in 0.01N HCl-MeOH	253 (6700), 322 (8500)	252 (5700), 327 (6500)
IR υ_{max}^{KBr} cm^{-1}	3450, 1710, 1670, 1600, 1520, 1460, 1340, 1230	3430, 1710, 1660, 1610, 1530 1340 1230
$[\alpha]_D^{25}$	+44.6° (*c* 0.51, MeOH)	+32.2° (*c* 0.23, MeOH)
Rf	0.28 [a]	0.52[a]
mp.	168 ~ 173 °C (dec.)	178 ~ 184 °C (dec.)

a: Silica gel TLC (Merck Art. No. 5715) $CHCl_3$:MeOH (10:1).

Kieselgel 60, 50 ml) using the mixture of toluene - acetone (10 : 1, 7 : 1, 5 : 1, 3 : 1 and 2 : 1) and further purified by rechromatography on a silica gel column (50 ml) using the mixture of toluene - acetone (50 : 1, 20 : 1, 10 : 1 and 7 : 1) to obtain the mixture of **1** and **2** (120 mg). The antibiotic mixture (35 mg) was separated by silica gel TLC (Merck, Art 105715, $CHCl_3$ - MeOH 20 : 1) providing 20 mg of **1** and 10 mg of **2**, respectively, as pale yellow powders.

Fig. 2. ORTEP drawing of **1**.



Physico-chemical properties of epoxyquinomicins A (**1**) and B (**2**) are summarized in Table 1. The antibiotics **1** and **2** are soluble in MeOH, AcOEt and acetone, slightly soluble in $CHCl_3$ and insoluble in *n*-hexane. The substances gave positive color reaction to molybdophosphoric acid-sulfuric acid, $FeCl_3$, Rydon-Smith and 2,4-dinitrophenylhydrazine reagents, and negative to ninhydrin reagent. The molecular formulae of **1** and **2** were determined to be $C_{14}H_{10}NO_6Cl$ and $C_{14}H_{11}NO_6$ by HRFABP-MS. The IR and UV spectra of **1** showed an epoxy-quinone structure at 1710 and 1670 cm^{-1} and 325 nm, respectively[1]. The IR and UV spectra of **2** were almost the same as those of **1**.

The structure of epoxyquinomicin A (**1**) was determined by X-ray analysis.† Compound **1** was recrystallized from MeOH solution to give yellow prism

Table 2. Crystal data of **1**.

Emprical formula	$C_{14}H_{10}O_6NCl$
Formula weight	323.69
Crystal system	orthorhombic
Space group	$P2_12_12_1$
Lattice Parameters:	a = 16.539(2) Å b = 17.805(2) Å c = 4.402(3) Å V = 1296.3(8) $Å^3$
Z	4
Dcalc.	1.66 g/cm^3
μ(CuKα)	29.4 cm^{-1}

Table 3. ^{13}C NMR data (100 MHz) and ^1H NMR data (400 MHz) of epoxyquinomicins A (**1**) and B (**2**) in CD_3OD[a].

	1		**2**	
Position	^{13}C	^1H	^{13}C	^1H
1	189.0		190.0	
2	141.4		141.7	
3	116.8	7.59 (1H,s)	116.4	7.61 (1H, s)
4	194.5		194.6	
5	62.4		62.4	
6	56.5	4.06 (1H,s)	56.5	4.05 (1H, s)
7	57.6	4.03 (1H, d, J=13.2 Hz) 4.14 (1H, d, J=13.2 Hz)	57.7	4.02 (1H, d, J=13.2 Hz) 4.14 (1H, d, J=13.2 Hz)
8	166.6		167.1	
9	121.5		119.3	
10	154.3		158.1	
11	123.9		117.9	6.97 (1H, d, J=8.3 Hz)
12	135.7	7.59 (1H, dd, J=1.5, 7.8 Hz)	135.8	7.44 (1H, m)
13	121.6	7.00 (1H, t, J=7.8 Hz)	121.3	6.99 (1H, td, J=1.5, 8.3 Hz)
14	131.0	7.98 (1H, dd, J=1.5, 7.8 Hz)	132.5	8.01 (1H, dd, J=1.5, 8.3 Hz)

a Chemical shifts in ppm from TMS as an internal satandard.

† A yellow prism crystal of $C_{14}H_{10}O_6NCl$ having approximate dimensions of 0.20 × 0.20 × 0.25 mm was mounted on a glass fiber. All measurements were made on a Rigaku AFC7R diffractometer with graphite monochromated Cu-Kα radiation. Of the 2748 reflections which were collected, 1361 were unique. No decay correction was applied. An empirical absorption correction using the program DIFABS[2] was applied which resulted in transmission factors ranging from 0.92 to 1.06. The structure was solved by direct methods (SHELXS86)[3] and expanded using Fourier techniques (DIRDIF92).[4] The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on 1303 observed reflections ($I > 2\sigma(I)$) and 200 variable parameters and converged with unweighted and weighted agreement factors of R = 0.029 and Rw = 0.047. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.16 and $-0.24\,e^-/Å^3$, respectively. Comparing |Fobs (hkl)|/|Fobs ($\bar{h}\bar{k}\bar{l}$)| and |Fcalc (hkl)|/|Fcalc ($\bar{h}\bar{k}\bar{l}$)| for 133 Friedel pairs for which the differences ||Fobs (hkl)| − |Fobs ($\bar{h}\bar{k}\bar{l}$)|| are greater than 1.0, 124 pairs showed consistently the absolute configuration in Fig. 2. All calculations were performed using the teXsan crystallographic software package of Molecular Structure Corporation.

Table 4. Antimicrobial activities of Jepoxyquinomicins A (**1**) and B (**2**).

Test organism	MIC (μg/ml)		Test organism	MIC (μg/ml)	
	1	**2**		**1**	**2**
Staphylococcus aureus FDA209P	12.5	12.5	*S. sonnei* JS11746	>100	100
S. aureus Smith	12.5	12.5	*Salmonella typhi* T-63	100	100
S. aureus MS9610	50	25	*S. enteritidis* 1891	>100	100
S. aureus No. 5 (MRSA)	25	25	*Proteus vulgaris* OX19	100	100
S. aureus No.17 (MRSA)	25	25	*P. mirabilis IFM* OM-9	50	50
Micrococcus luteus FDA16	12.5	25	*Providencia rettgeri* GN311	100	100
M. luteus IFO3333	3.12	6.25	*P. rettgeri* GN466	100	100
M. luteus PCI1001	6.25	6.25	*Serratia marcescens*	100	100
Bacillus anthracis	25	12.5	*Pseudomonas aeruginosa A3*	>50	>50
B. subtilis NRRL B-558	50	12.5	*Pasteurella piscicida* sp.6395	12.5	12.5
B. subtilis PCI219	12.5	6.25	*P. piscicida* sp.6356	12.5	6.25
B. cereus ATCC10702	25	12.5	*P. piscicida* p-3340	6.25	12.5
Corynebacterium bovis 1810	50	50	*P. piscicida* p-3343	6.25	12.5
Escherichia coli NIHJ	50	50	*P. piscicida* p-3344	6.25	12.5
E. coli K-12	100	100	*P. piscicida* p-3346	6.25	6.25
E.coli K-12 MLI629	>100	100	*P. piscicida* p-3347	3.12	12.5
E. coli BEM11	50	50	*P. piscicida* p-3348	3.12	12.5
E. coli BE1121	50	50	*P. piscicida* p-3349	3.12	12.5
E. coli BE1186	50	50	*P. piscicida* p-3350	6.25	12.5
Shigella dysenteriae JS11910	50	50	*P. piscicida* p-3353	3.12	12.5
S. flexneri 4b JS11811	100	100	*P. piscicida* p-3354	6.25	12.5

crystals. As a result, the absolute stereochemistry of **1** was confirmed to be (5*R*,6*S*)-2-(3-chloro-2-hydroxybenzoylamino)-5-hydroxymethyl-5,6-epoxy-2-cyclohexene-1,4-dione. The ORTEP drawing of **1** is shown in Fig. 2. Crystal data are summarized in Table 2. The ^{13}C and ^{1}H NMR spectral data of **1** and **2** are summarized in Table 3. All the assignments of protons and carbons of **1** were confirmed by various NMR spectral analyses including ^{1}H-^{1}H COSY, DEPT, HMQC and HMBC of **1**. The structure of **2** was deduced to be a dechlorinated derivative of **1** from NMR spectral data and from the molecular formula of **2** (Fig. 2).

As shown in Table 4, antimicrobial activities of epoxyquinomicins A (**1**) and B (**2**) are moderate against Gram-positive bacteria and several strains of *Pasteurella piscicida*.

Toshio Tsuchida
Maya Umekita
Naoko Kinoshita
Hironobu Iinuma
Hikaru Nakamura
Kazuo T. Nakamura†
Hiroshi Naganawa
Tsutomu Sawa

Masa Hamada
Tomio Takeuchi

Institute of Microbial Chemistry,
3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141, Japan
† School of Pharmaceutical Sciences, Showa University,
Hatanodai, Shinagawa-ku, Tokyo 142, Japan

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