Epoxyquinomicins A, B, C and D, New Antibiotics from *Amycolatopsis*

III. Physico-chemical Properties and Structure Determination

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The structures of epoxyquinomicins A (**1**), B (**2**), C (**3**) and D (**4**) were determined by spectroscopic studies. Compound **1** was determined to be (5*R*,6*S*)-2-(3-chloro-2-hydroxybenzoylamino)-5-hydroxymethyl-5,6-epoxy-2-cyclohexene-1,4-dione. Compound **2** was revealed to be the dechlorinated derivative of **1**. Compounds **3** and **4** were determined to be the reduced derivative of **2** and **1**, respectively.

In the accompanying papers[1,2], we described the taxonomy of the producing strain, fermentation, isolation and biological activities of epoxyquinomicins A (**1**), B (**2**), C (**3**) and D (**4**) (Fig. 1), isolated from the culture broth of *Amycolatopsis* sp. MK299-95F4. All these antibiotics show anti-inflammatory activity and low toxicity. In particular, Compounds **3** and **4** have less cytotoxicity, acute toxicity and antimicrobial activity. In this paper, we describe physico-chemical properties and structure determination of those antibiotics named as epoxyquinomicins.

Results

Physico-chemical Properties

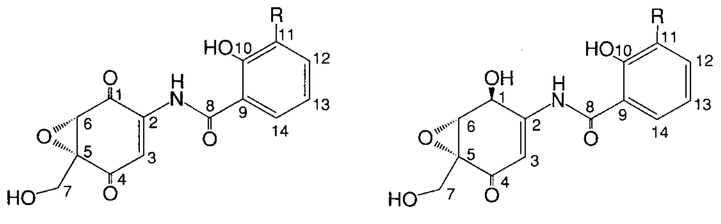
Physico-chemical properties of **1**, **2**, **3** and **4** were summarized in Table 1. They are of acidic natures, soluble in methanol, acetone and ethyl acetate, slightly soluble in chloroform and insoluble in *n*-hexane. The molecular formulae of **1**, **2**, **3** and **4** were determined by HRFAB-MS to be $C_{14}H_{10}NO_6Cl$, $C_{14}H_{11}NO_6$, $C_{14}H_{13}NO_6$ and $C_{14}H_{12}NO_6Cl$, respectively. The IR spectra of **1**, **2**, **3** and **4** showed characteristic absorption bands of an amide carbonyl group at 1640～1670 cm^{-1} and hydroxyl group at 3430～3450 cm^{-1}. UV spectra of **1** and **3** are essentially the same pattern as those of **2** and **4**, respectively. Compounds **1**～**4** gave positive color reactions to molybdophosphoric acid-sulfuric acid, $FeCl_3$, Rydon-Smith and 2,4-dinitrophenylhydrazine reagents, and negative to ninhydrin reagent.

The ^1H (500 MHz) and ^{13}C (125 MHz) NMR data in CD_3OD are summarized in Table 2.

Structure of Epoxyquinomicin D

The molecular formula of **4** suggested that **4** was highly oxidated having nine double bonds or its equivalents. The ^1H and ^{13}C NMR spectra of **4** showed the presence of eight protons and fourteen carbon signals, respectivery, in consistent with the molecular formula. The carbon atoms in **4** are classified into one methylene, two sp^3 methines, one sp^3 quarternary carbon, four sp^2 methines, six quarternary carbons including two carbonyl carbons by thier chemical sifts and the DEPT

Fig. 1. Structures of epoxyquinomicins A, B, C and D.



Epoxyquinomicin A (**1**): R = Cl
Epoxyquinomicin B (**2**): R = H

Epoxyquinomicin C (**3**): R = H
Epoxyquinomicin D (**4**): R = Cl

Table 1. Physico-chemical properties of epoxyquinomicins A (**1**), B (**2**), C (**3**) and D (**4**).

	1	**2**	**3**	**4**
Appearance	Pale yellow powder	Pale yellow powder	White powder	White powder
Nature	Acidic	Acidic	Acidic	Acidic
Molecular formula	$C_{14}H_{10}NO_6Cl$	$C_{14}H_{11}NO_6$	$C_{14}H_{13}NO_6$	$C_{14}H_{12}NO_6Cl$
FAB-MS (*m/z*)	$(M+H)^+$ 324 $(M-H)^-$ 322	M^+ 289 $(M-H)^-$ 288	$(M+H)^+$ 292 $(M-H)^-$ 290	$(M+H)^+$ 326 $(M-H)^-$ 324
HRFAB-MS (*m/z*)	Calcd: 322.0118 (as $C_{14}H_9NO_6Cl$) Found: 322.0136 $(M-H)^-$	Calcd: 290.0664 (as $C_{14}H_{12}NO_6$) Found: 290.0656 $(M+H)^+$	Calcd: 292.0804 (as $C_{14}H_{14}NO_6$) Found: 292.0821 $(M+H)^+$	Calcd: 326.0417 (as $C_{14}H_{13}NO_6Cl$) Found: 326.0431 $(M+H)^+$
UV λ_{max} (ε) in MeOH	236 (sh, 8900), 255 (sh, 5900), 325 (8000), 370 (sh, 2700)	237 (6100), 253 (sh, 5400), 326 (6300)	297 (17430)	299 (17590)
in 0.01 N NaOH-MeOH	234 (sh, 11200), 257 (sh, 5100), 327 (8300), 371 (sh, 4400)	235 (9100), 259 (sh, 4000), 324 (5800), 376 (sh, 3400)	304 (18270), 364 (9750)	304 (18950), 367 (9230)
in 0.01 N HCl-MeOH	253 (6700), 322 (8500)	252 (5700), 327 (6500)	296 (18140)	297 (18530)
$[\alpha]_D^{25}$ (MeOH)	+44.6° (*c* 0.51)	+32.2° (*c* 0.23)	+128° (*c* 1.0)	+142° (*c* 1.0)
IR ν_{max} (KBr) cm^{-1}	3450, 1710, 1670, 1600, 1520, 1460, 1340, 1230	3430, 1710, 1660, 1610, 1530, 1340, 1230	3431, 1660, 1604, 1537, 1460, 1309, 1232, 1065, 750	3438, 1643, 1533, 1281, 1200
Rf[a]	0.63	0.76	0.61	0.49
mp (°C)	168~173 (dec.)	178~184 (dec.)	168~172 (dec.)	163~168 (dec.)

[a] Silica gel TLC (Merck Art. 1.05715, toluene : acetone = 1 : 2).

Table 2. ^1H and ^{13}C NMR chemical shifts of epoxyquinomicins A (**1**), B (**2**), C (**3**) and D (**4**) in CD_3OD.

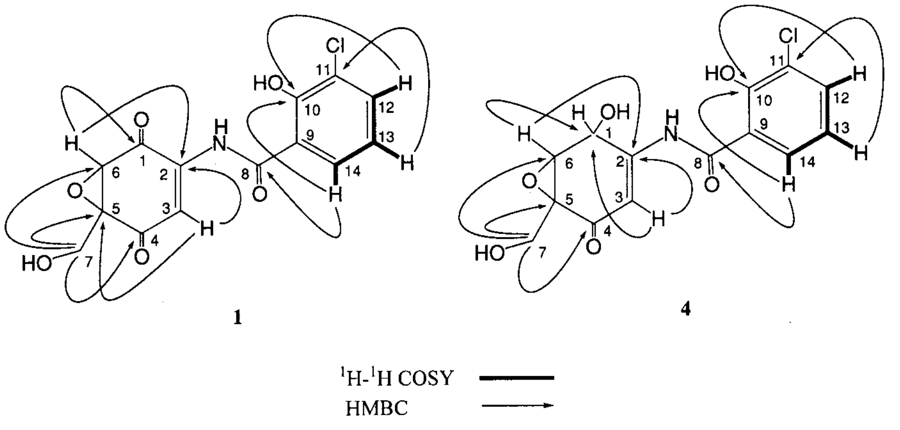
Position	**1**		**2**		**3**		**4**	
	δ_C[a]	δ_H[b]	δ_C[a]	δ_H[b]	δ_C[a]	δ_H[b]	δ_C[a]	δ_H[b]
1	189.0		190.0		66.2	4.68 (s)	65.8	4.78 (d, 1.5)
2	141.4		141.7		153.0		152.8	
3	116.8	7.59 (s)	116.4	7.61 (s)	110.0	7.04 (s)	110.6	6.98 (s)
4	194.5		194.6		196.5		196.5	
5	62.4		62.4		59.4		59.5	
6	56.5	4.06 (s)	56.5	4.05 (s)	59.7	3.85 (s)	59.7	3.88 (d, 1.5)
7	57.6	4.03 (d, 13.2)	57.7	4.02 (d, 13.2)	58.7	3.92 (d, 13.2)	58.7	3.93 (d, 13.2)
		4.14 (d, 13.2)		4.14 (d, 13.2)		4.05 (d, 13.2)		4.06 (d, 13.2)
8	166.6		167.1		168.0		168.8	
9	121.5		119.3		119.1		120.5	
10	154.3		158.1		158.4		155.7	
11	123.9		117.9	6.97 (d, 8.3)	118.0	6.99 (d, 7.8)	123.8	
12	135.7	7.59 (dd, 1.5, 7.8)	135.8	7.44 (m)	135.7	7.43 (dd, 1.5, 7.8)	135.7	7.58 (dd, 1.5, 7.8)
13	121.6	7.00 (t, 7.8)	121.3	6.99 (dt, 8.3, 1.5)	121.3	6.98 (t, 7.8)	121.2	6.97 (t, 7.8)
14	131.6	7.98 (dd, 1.5, 7.8)	132.5	8.01 (dd, 8.3, 1.5)	131.9	7.97 (dd, 1.5, 7.8)	129.5	7.88 (dd, 1.5, 7.8)

[a] Chemical shifts in ppm.

[b] Chemical shifts in ppm (multiplicity, coupling constants in Hz).

spectrum. The connectivity of proton and carbon atoms were confirmed by the HMQC spectrum. The proton sequence from H-12 to H-14 *via* H-13 was analyzed by the ^1H-^1H COSY spectrum. And the long-range couplings from H-12 (δ 7.58) to C-10 (δ 155.7), from H-13 (δ 6.97) to C-11 (δ 123.8) and from H-14 (δ 7.88) to C-10 and C-8 carbonyl (δ 168.8) were observed in the HMBC spectrum. These evidences suggested a substituted salicyloyl moiety was in **4**. The long-range couplings from methylene proton H-7 (δ 3.93, 4.06) to C-5 (δ 59.5), C-6 (δ 59.7) and C-4 carbonyl (δ 196.5), from H-3 (δ 6.98) to C-1 (δ 65.8) and C-2 (δ 152.8), from H-6 (δ 3.88) to C-1 and C-2 indicated the presence of a 4-hydroxy-6-hydroxymethyl-2-cyclohexene-1-one moiety. The carbon signals for C-5 and C-6 were assignable to an epoxy ring from their chemical shifts and the spin coupling constant between C-6 and H-6 which was observed to be 184.2 Hz by a proton non-decoupled INEPT experiment.

Fig. 2. Observed correlations in the ^{1}H-^{1}H COSY and HMBC experiments of **1** and **4**.



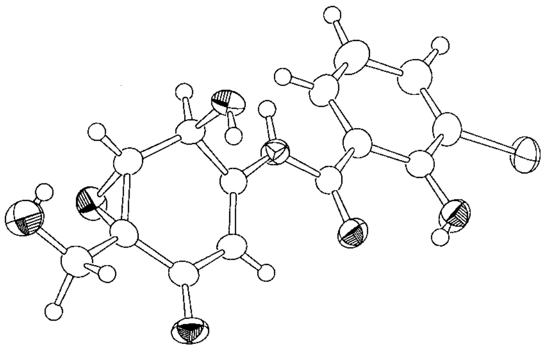
1 **4**

Since an amide group was suggested by the IR spectrum of **4** and positive color reaction for Rydon-Smith reagent, a nitrogen atom could be inserted between C-2 and C-8 carbons. Remaining chlorine atom was able to place at C-11 to accomplish the structure of **4** (Fig. 2).

Absolute stereochemistry of **4** was established by X-ray analysis of prism crystal which was obtained from acetone - toluene (1 : 3) solution. The ORTEP drawing of **4** is shown in Fig. 3.

As a result, **4** was confirmed to be (4*S*,5*R*,6*R*)-3-(3-chloro-2-hydroxybenzoylamino)-4-hydroxy-6-hydroxymethyl-5,6-epoxy-2-cyclohexene-1-one.

Fig. 3. The ORTEP drawing of epoxyquinomicin D (**4**).



Structures of Epoxyquinomicins A, B and C

Compound **3** was revealed to be the dechlorinated derivative of **4** by the comparison of the ^{1}H and ^{13}C NMR spectral data and molecular formula of **3** with those of **4**.

The structures of **1** and **2** were determined by various NMR analyses and X-ray analysis[3]. Each compounds **1** and **2** possesses a carbonyl group at C-1 position instead of a hydroxyl group in **4** and **3**, respectively.

Discussion

The structures of **1**, **2**, **3** and **4**, which were isolated from the culture broth of *Amycolatopsis* sp. MK299-95F4, were determined by spectroscopic studies. Compounds **1**, **2**, **3** and **4** showed improvement of collagen induced arthritis *in vivo*. Compound **1** has been determined to be (5*R*,6*S*)-2-(3-chloro-2-hydroxybenzoylamino)-5-hydroxymethyl-5,6-epoxy-2-cyclohexene-1,4-dione. Compound **2** was the dechlorinated derivative of **1**. In this paper, **3** and **4** were revealed to be the reduced derivative of **2** and **1**, respectively. Structurally-related natural products such as manumycin[4], alisamycin[5], asukamycin[6] isolated from the culture broth of *Streptomyces* sp. have been reported. They have 5,6-epoxy-4-hydroxy-2-cyclohexene-1-one moiety in their structures and these compounds also display attractive biological properties such as Ras farnesyltransferase inhibitory activity in addition to their antibacterial, antifungal or antitumor activities. Recently, EI-1507-1, -2[7], EI-1511-3, -5 and EI-1625-2[8] were reported to show inhibitory activity against IL-1β converting enzyme.

It was supposed that antibiotics having 5,6-epoxy-2-cyclohexene-1,4-dione or 5,6-epoxy-4-hydroxy-2-cyclohexene-1-one moiety in the molecule might display interesting activities. Synthetic studies on epoxyquinomicins are now in progress.

Experimental

Spectroscopic Methods

^1H and ^{13}C NMR spectra were recorded on a JEOL JNM-A500 and a JEOL JNM-EX400 spectrometers. Chemical shifts are given in ppm from TMS as an internal standard. FAB-MS and HRFAB-MS were obtained on a JEOL JMS-SX102 mass spectrometer. UV absorption spectra were measured with a Hitachi U-3210 spectrophotometer. IR absorption spectra were obtained with a Hitachi I-5020 FT-IR spectrometer. Optical rotations were taken by a Perkin-Elmer 241 polarimeter using a micro-cell (light path 10 cm). MP's were determined with a Yanaco MP-S3 micro melting point apparatus, which were uncorrected.

X-Ray Crystallography for 4

A pale yellow prism crystal of 0.01 × 0.05 × 0.35 mm was mounted on a glass fiber. All measurements were made on a Rigaku AFC7R diffractometer with graphite monochromated Cu-Kα radiation. Cell constants and an orientation matrix for data collection were obtained from a least-squares refinement using the setting angles of 22 carefully centered reflections in the range $50.32 < 2\theta < 57.68°$. Crystal data are as follows: Empirical formula; $C_{14}H_{12}NO_6Cl$. Formula weight; 325.70. Crystal system; monoclinic. Space group; $P2_1$. Lattice parameters; $a = 8{,}756(1)$Å, $b = 6.7999(6)$Å, $c = 11.2953(8)$Å, $\beta = 93.362(7)°$, $V = 671.3(1)$Å^3. Z value; 2. Dcalc; 1.611 g/cm^3. μ (CuKα); 28.34 cm^{-1}. The reflection data were collected at a temperature of 21°C using the ω-2θ scan technique to a maximum 2θ value of 130.2°. Scans of $(0.73 + 0.30 \tan\theta)°$ were made at a speed of 8.0°/minute (in omega). Of the 2264 reflections which were collected, 1249 were unique. The intensities of three standard reflections were measured after every 150 reflections, no significant intensity decay was observed. An absorption correction using the program DIFABS[9)] was applied which resulted in transmission factors ranging from 0.74 to 1.00. The data were collected for Lorentz and polarization effects. The structure was solved by a direct method (SHELXS86)[10)]. 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 1140 observed reflections ($I > 2\sigma(I)$) and 198 variable parameters and converged with unweighed and weighted agreement factors of $R = 0.038$ and $Rw = 0.049$. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.19 and $-0.31e^-/$Å^3, respectively. Comparing $|F_o(hkl)|/|F_o(\bar{h}\bar{k}\bar{l})|$ and $|F_c(hkl)|/|F_c(\bar{h}\bar{k}\bar{l})|$ for 243 Bijvoet mates for which the difference $||F_c(hkl)| - |F_c(\bar{h}\bar{k}\bar{l})||/\sqrt{\sigma^2F_o(hkl) + \sigma^2F_o(\bar{h}\bar{k}\bar{l})}$ are larger than 1.0, 236 pairs showed consistently the absolute configuration in Fig. 3. All calculations were performed using the teXsan crystallographic software package of Molecular Structure Corporation.

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