Pyralomicins, Novel Antibiotics from *Microtetraspora spiralis*

IV. Absolute Configuration

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The absolute configurations of pyralomicin 1a and pyralomicin 2a were determined by X-ray crystallographic analyses of crystalline 7′-*O*-*p*-bromobenzoylpyralomicin 1a and 2′-*O*-*p*-bromobenzoylpyralomicin 2a derived from pyralomicin 1a and pyralomicin 2a using anomalous scattering of the bromine atom. The absolute configurations of pyralomicins 1b～1d and 2b～2c were suggested to be the same as pyralomicin 1a and pyralomicin 2a, respectively, by comparing the circular dichroism spectra.

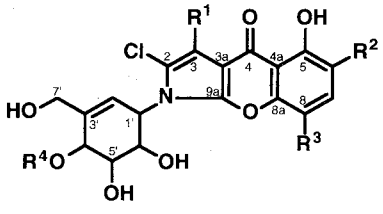
We have isolated novel antibiotics, pyralomicins 1a～1d (**1**～**4**), and 2a～2c (**5**～**7**) (Fig. 1) from the culture broth of *Microtetraspora spiralis* MI178-34F18[1], and elucidated the planar structures and relative configurations by NMR spectroscopy[2]. In this paper, we describe the absolute configurations of **1**～**7**.

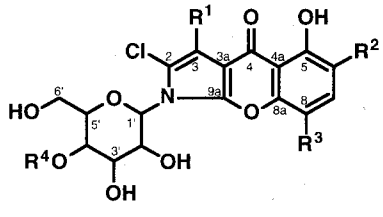
Results and Discussion

Pyralomicins (**1**～**7**) are crystalline compounds but their X-ray crystallographic analyses gave no solutions for absolute stereochemistry due to their microcrystalline nature. By *p*-bromobenzoylation of **1** which was a major component of 1 series in pyralomicins (**1**～**4**) and the separation of random *p*-bromobenzoylated products, crystalline 7′-*O*-*p*-bromobenzoylpyralomicin 1a (**8**) was obtained. In the same manner, crystalline 2′-*O*-*p*-bromobenzoylpyralomicin 2a (**9**) was obtained. The positions of the *p*-bromobenzoyl groups in **8** and **9** were determined to be 7′ and 2′ by the analyses of NMR spectra, respectively. Both compounds were applied to X-ray crystallography and they gave solutions. The conditions of the X-ray crystallographic analyses are described in Table 1, and the absolute configurations were established to be as shown in Fig. 2 and Fig. 3 using anomalous scattering of the bromine atom. The absolute structures of **1** and **5** were determined to be 2,6-dichloro-1-[(1*R*,4*R*,5*S*,6*S*)-5,6-dihydroxy-3-(hydroxymethyl)-4-methoxy-2-cyclohexen-1-yl]-5-hydroxy-8-methyl-[1]-benzopyrano[2,3-b]pyrrole-4-(1*H*)-one and 2,6-dichloro-5-hydroxy-8-methyl-1-(4-*O*-methyl-β-D-glucopyranosyl)-[1]benzopyrano[2,3-b]-pyrrole-4-(1*H*)-one, respectively.

As described in our previous paper[2], the relative configurations of the 1 series (**1**～**4**) and the 2 series (**5**～**7**) are the same, respectively. To establish the

Fig. 1. Planar structures of pyralomicins (**1**～**7**).





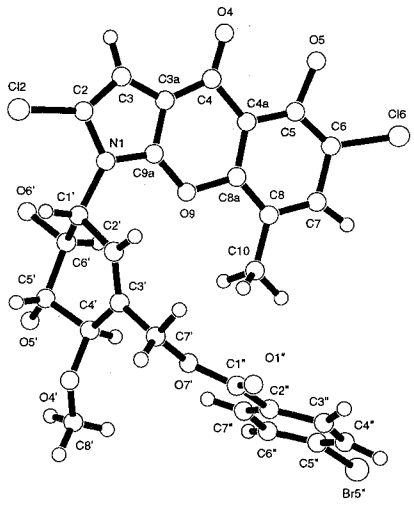
	R^1	R^2	R^3	R^4
Pyralomicin 1a (**1**)	H	Cl	CH_3	CH_3
Pyralomicin 1b (**2**)	H	CH_3	Cl	CH_3
Pyralomicin 1c (**3**)	H	Cl	CH_3	H
Pyralomicin 1d (**4**)	Cl	Cl	CH_3	H

	R^1	R^2	R^3	R^4
Pyralomicin 2a (**5**)	H	Cl	CH_3	CH_3
Pyralomicin 2b (**6**)	H	CH_3	Cl	CH_3
Pyralomicin 2c (**7**)	H	Cl	CH_3	H

Table 1. Crystal data of 7′-*O*-*p*-bromobenzoylpyralomicin 1a (**8**) and 2′-*O*-*p*-bromobenzoylpyralomicin 2a (**9**).

	8	**9**
Formula	$C_{27}H_{22}NO_8Cl_2Br$	$C_{26}H_{22}NO_9Cl_2Br$
Formula weight	639.28	643.27
Crystal system	Orthorhombic	Monoclinic
Cell data		
a	29.449(4) Å	15.130(4) Å
b	6.946(8) Å	21.827(3) Å
c	14.328(4) Å	17.261(4) Å
β		99.94(2)°
V	2931(2) Å^3	5614(2) Å^3
Space group	$P2_12_12$	$P2_1$
Z value	4	8
D_{calc}	1.449 g/cm^3	1.522 g/cm^3
μ (CuKα)	40.05 cm^{-1}	42.12 cm^{-1}

Fig. 2. Molecular structure of **8**.



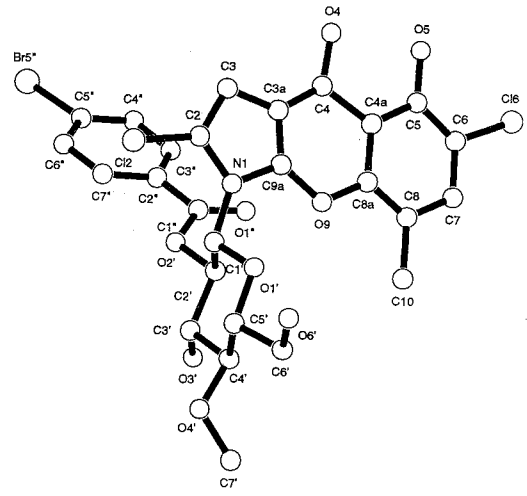
absolute configurations of **2**～**4** and **6**～**7**, we measured their circular dichroism (CD) spectra in methanol. The CD spectra of **2**～**4** were similar to that of **1**; (**1**: θ_{206}, −53000, θ_{253}, −12000, θ_{359}, −2300, **2**: θ_{206}, −47000, θ_{252}, −10000, θ_{360}, −1900, **3**: θ_{205}, −47000, θ_{252}, −11000, θ_{350}, −1800, **4**: θ_{206}, −49000, θ_{255}, −13000, θ_{349}, −1900) and the spectra of **6**～**7** were also similar to that of **5**; (**5**: θ_{208}, −1700, θ_{244}, 4800, θ_{293}, 1400, θ_{358}, −1200, **6**: θ_{210}, −2600, θ_{243}, 5500, θ_{285}, 500, θ_{352}, −540, **7**: θ_{210}, −1500, θ_{244}, 3700, θ_{287}, 1100, θ_{352}, −1200). Therefore, the absolute configurations of **2**～**4** and **6**～**7** were suggested to be the same as **1** and **5**, respectively.

Experimental

General

MPs were determined on a Yanagimoto micro melting point apparatus and were uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. NMR spectra were recorded on a JEOL JNM-A500 spectrometer. Mass spectra were taken by a JEOL JMS-SX102 spectrometer. CD spectra were obtained with a JASCO J-720 spectrometer (solvent: methanol; concentration: 10 μg/ml; temperature: 25°C).

Fig. 3. Molecular structure of **9**.



7′-*O*-*p*-Bromobenzoylpyralomicin 1a (**8**)

Pyralomicin 1a (**1**) (130 mg) in pyridine (10 ml) was treated with *p*-bromobenzoyl chloride (100 mg × 3 at 0, 24 and 48th hour) for 72 hours at room temperature. The reaction mixture was then evaporated under reduced pressure; the residue was dissolved in ethyl acetate, and the solution was washed with aqueous sodium hydrogen carbonate and water, and dried over anhydrous magnesium sulfate. After removal of the solvent, the residue was purified by silica gel column chromatography (Wakogel C-300 (Wako Pure Chemical Industries, Ltd., Japan), *n*-hexane - ethyl acetate 2 : 1, 1 : 1, 1 : 2, 1 : 4 stepwise). Four major products (Rf 0.67, 0.62, 0.43, 0.34, silica gel TLC (Merck, Kiesel gel 60F_{254}, ethyl acetate)) were isolated and one of them, compound **8** whose Rf value was 0.62, gave crystals (prisms) from dichloromethane-*n*-hexane solution. Physico-chemical properties of **8**: FAB-MS *m/z* 638 (MH^+, $C_{27}H_{23}NO_8Cl_2Br$); MP 173～175°C; $[\alpha]_D^{26}$ −192.5° (*c* 0.2, *N*,*N*-dimethylformamide); Rf 0.62 (silica gel TLC (Merck, Kiesel gel 60F_{254}, ethyl acetate); ^{1}H NMR (500 MHz, *N*,*N*-dimethylformamide-d_7) δ 2.38 (3H, s, 8-CH_3), 3.63 (3H, s, 4′-OCH_3), 3.98 (1H, dd, J=9.8, 7.7 Hz, 5′-H), 4.28 (1H, d, J=7.7 Hz, 4′-H), 4.30 (1H, t, J=9.8 Hz, 6′-H), 4.91 (1H, d, J=13.1 Hz, 7′-H_A), 5.12 (1H, d, J=13.1 Hz, 7′-H_B), 5.22 (1H, br d, 1′-H), 5.53 (1H, br d, OH), 5.83 (1H, br s, OH), 6.20 (1H, br s, 2′-H), 6.73 (1H, s, 3-H), 7.67 (1H, s, 7-H), 7.79 (2H, d, J=8.5 Hz, *p*-bromobenzoyl group), 8.00 (2H, d, J=8.5 Hz, *p*-bromobenzoyl group), 13.81 (1H, br s, 5-OH).

2′-*O*-*p*-Bromobenzoylpyralomicin 2a (**9**)

Pyralomicin 2a (**5**) (130 mg) in pyridine (10 ml) was treated with *p*-bromobenzoyl chloride (200 mg) for 24 hours at room temperature. After the same workup procedure as described in the preparation of **8**, the reaction mixtures were purified by silica gel column chromatography (Wakogel C-300 (Wako Pure Chemical Industries, Ltd., Japan), chloroform - methanol, 30 : 1, 20 : 1 stepwise). Three major products (Rf 0.47, 0.15, 0.12, silica gel TLC (Merck, Kiesel gel $60F_{254}$, chloroform - methanol 30 : 1)) were isolated and one of them, compound **9** whose Rf value was 0.15, gave crystals (prisms) from dichloromethane-*n*-hexane solution. Physico-chemical properties of **9**: FAB-MS *m/z* 642 (MH^+, $C_{26}H_{23}NO_9Cl_2Br$); MP 203~206°C; $[\alpha]_D^{24}$ −174.0° (*c* 0.2, *N*,*N*-dimethylformamide); Rf. 0.15 (silica gel TLC Merck, Kiesel gel $60F_{254}$, chloroform - methanol 30 : 1); ^1H NMR (500 MHz, *N*,*N*-dimethylformamide-d_7) δ 2.61 (3H, s, 8-CH_3), 3.63 (1H, t, J=9.2 Hz, 4′-H), 3.67 (3H, s, 4′-OCH_3), 3.81 (1H, m, 6′-H_A), 3.90 (1H, m, 6′-H_B), 3.90 (1H, m, 5′-H), 4.31 (1H, t, J=9.2 Hz, 3′-H), 5.10 (1H, br d, 6′-OH), 5.95 (1H, dd, J=9.5, 9.2 Hz, 2′-H), 6.05 (1H, d, J=9.5 Hz, 1′-H), 6.19 (1H, br s, 3′-OH), 6.67 (1H, s, 3-H), 7.76 (2H, d, J=8.5 Hz, *p*-bromobenzoyl group), 7.82 (1H, s, 7-H), 7.82 (2H, d, J=8.5 Hz *p*-bromobenzoyl group), 13.58 (1H, br s, 5-OH).

X-Ray Crystallography of **8**

A yellow prism crystal of **8** ($C_{27}H_{22}NO_8Cl_2Br$) having approximate dimensions of 0.02 × 0.08 × 0.35 mm was mounted on a glass fiber. All measurements were made on a Rigaku AFC-7R diffractometer with graphite monochromated Cu-Kα radiation. Of the 3786 reflections which were collected, 2550 were unique. No decay correction was applied. An empirical absorption correction using the program DIFABS[3] was applied which resulted in transmission factors ranging from 0.51 to 1.24. The structure was solved by direct methods[4] and expanded using Fourier techniques[5]. 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 1023 observed reflections ($I > 1.50\,s(I)$) and 353 variable parameters and converged with unweighted and weighted agreement factors of R=0.063 and Rw=0.070. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.39 and −0.30 $e^-/Å^3$, 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 170 FRIEDEL pairs for which the differences $||F_C(hkl)|-|F_C(\bar{h}\bar{k}\bar{l})||$ are greater than 1.0, 129 pairs showed consistently the absolute configuration in Fig. 2. All calculations were performed using the teXsan[6] crystallographic software package of Molecular Structure Corporation.

X-Ray Crystallography of **9**

A pale yellow prism crystal of **9** ($C_{26}H_{22}NO_9Cl_2Br$) having approximate dimensions of 0.15 × 0.15 × 0.20 mm was mounted on a glass fiber. All measurements were made on a Rigaku AFC-7R diffractometer with graphite monochromated Cu-Kα radiation. Of the 13071 reflections which were collected, 8602 were unique. No decay correction was applied. An empirical absorption correction using the program DIFABS[3] was applied which resulted in transmission factors ranging from 0.75 to 1.00. The structure was solved by Patterson methods and expanded using Fourier techniques[5]. Some non-hydrogen atoms were refined anisotropically, while the rest were refined isotopically. The final cycle of full-matrix least-squares refinement was based on 5612 observed reflections ($I > 2.00\,s(I)$) and 1341 variable parameters and converged with unweighted and weighted agreement factors of R=0.098 and Rw=0.128. 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 1474 FRIEDEL pairs for which the differences $||F_C(hkl)|-|F_C(\bar{h}\bar{k}\bar{l})||$ are greater than 1.0, 1210 pairs showed consistently the absolute configuration in Fig. 3. All calculations were performed using the teXsan[6] crystallographic software package of Molecular Structure Corporation.

Acknowledgment

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