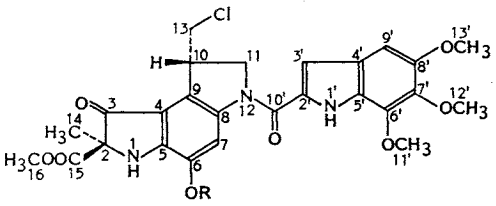
PYRINDAMYCINS A AND B, NEW ANTITUMOR ANTIBIOTICS

Sir:

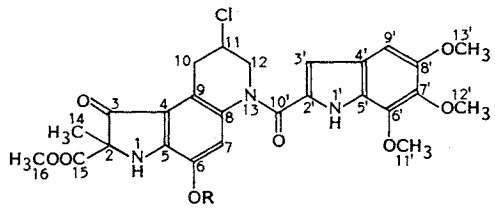
Pyrindamycins A (**1**) and B (**2**), two new potent antitumor antibiotics related to CC-1065[1~3] have been found in the culture broth of *Streptomyces* sp. SF2582, which was isolated from a soil sample collected at Sagamihara, Kanagawa Prefecture, Japan. The antibiotics are active against Gram-positive and Gram-negative bacteria, and exhibit strong therapeutic effects against both drug-sensitive and resistant cells of P388 leukemia in mice. In this communication, the isolation, characterization and structural elucidation of the antibiotics are reported.

Fermentation of *Streptomyces* sp. SF2582 was



1 R = H

4 R = $COCH_3$



2 R = H

5 R = $COCH_3$

Table 1. ^1H NMR chemical shifts.

Proton	Chemical shifts ppm (*J*, Hz)				
	1	**2**	**3**	**4**	**5**
NH	5.62 (1H, s)	5.36 (1H, s)		5.22 (1H, s)	5.19 (1H, s)
6-OH	10.16 (1H, br s)				
6-$OCOCH_3$				2.38 (3H, s)	2.30 (3H, s)
7-H	8.53 (1H, s)	7.08 (1H, s)		8.48 (1H, s)	7.46 (1H, s)
10-H	4.08 (1H, m)	3.50 (1H, dd, *J*=19.3, 3.4), 3.60 (1H, dd, *J*=19.3, 6.0)		4.18 (1H, m)	3.58 (1H, dd, *J*=19.5, 3.9), 3.71 (1H, dd, *J*=19.5, 5.5)
11-H	4.52 (1H, dd, *J*=10.8, 5.0), 4.57 (1H, dd, *J*=10.8, 8.7)	4.49 (1H, m)		4.64 (2H, d, *J*=6.6)	4.50 (1H, m)
12-H		4.00 (1H, m), 4.58 (1H, d, *J*=10.0)			4.19 (1H, d, *J*=10.2), 4.50 (1H, m)
13-H	3.70 (1H, dd, *J*=10.5, 8.5), 4.11 (1H, dd, *J*=10.5, 3.3)			3.76 (1H, m), 4.15 (1H, m)	
14-H	1.69 (3H, s)	1.64 (3H, s)		1.68 (3H, s)	1.68 (3H, s)
16-H	3.67 (3H, s)	3.74 (3H, s)		3.78 (3H, s)	3.79 (3H, s)
1′-H	9.66 (1H, br s)	9.39 (1H, br s)		9.46 (1H, br s)	9.20 (1H, br s)
3′-H	6.96 (1H, d, *J*=2.3)	6.63 (1H, br s)	6.90 (1H, s)	6.95 (1H, d, *J*=2.3)	6.58 (1H, br s)
9′-H	6.79 (1H, s)	6.71 (1H, s)	6.87 (1H, s)	6.86 (1H, s)	6.76 (1H, s)
11′-H	4.06 (2H, s)	3.99 (3H, s)	4.04 (3H, s)	4.06 (3H, s)	4.07 (3H, s)
12′-H	3.94 (3H, s)	3.89 (3H, s)	3.87 (3H, s)	3.94 (3H, s)	3.93 (3H, s)
13′-H	3.89 (3H, s)	3.82 (3H, s)	3.86 (3H, s)	3.90 (3H, s)	3.90 (3H, s)

1, **2**, **4** and **5** in $CDCl_3$. **3** in CD_3OD.

Table 2. ^{13}C NMR chemical shifts.

Carbon	1	2	3	4	5
C-2	71.1 s	71.0 s		71.1 s	71.1 s
C-3	196.6 s	196.8 s		196.3 s	196.0 s
C-4	112.4 s	116.6[b]s		117.7 s	118.7 s
C-5	137.6 s	141.6[e]s		137.2 s	135.1 s
C-6	150.2 s	151.6 s		150.5 s	152.1 s
C-7	115.5 d	118.0 d		120.4 d	126.6 d
C-8	144.1 s	129.1[e]s		138.5 s	130.4 s
C-9	119.5 s	116.7[b]s		126.7 s	124.2 s
C-10	42.2 d	33.1 t		42.6 d	33.7 t
C-11	54.9 t	53.6 d		54.4 t	53.6 d
C-12		52.5 t			51.7 t
C-13	46.3 t			45.8 t	
C-14	21.9 q	21.8 q		22.0 q	22.1 q
C-15	169.5 s	169.6 s		169.1 s	169.2 s
C-16	53.3 q	53.4 q		53.6 q	52.7 q
C-2′	129.0[a]s	128.8[d]s	133.5[e]s	129.4[g]s	128.5[h]s
C-3′	107.8 d	108.1 d	104.7 d	106.5 d	107.8 d
C-4′	123.4[a]s	123.0[d]s	123.2[e]s	123.6[g]s	123.1[h]s
C-5′	125.9 s	125.9 s	124.3 s	125.5 s	125.8 s
C-6′	138.6 s	138.8 s	138.0[f]s	138.8 s	138.9 s
C-7′	140.7 s	140.2 s	138.2[f]s	140.6 s	140.4 s
C-8′	150.1 s	150.0 s	148.1 s	150.2 s	150.2 s
C-9′	97.7 d	97.6 d	97.3 d	97.7 d	97.6 d
C-10′	160.5 s	164.7 s	167.3 s	159.8 s	163.4 s
C-11′	61.2 q	61.1 q	59.3 q	61.1 q	61.1 q
C-12′	61.5 q	61.4 q	59.6 q	61.4 q	61.4 q
C-13′	56.3 q	56.2 q	54.6 q	56.3 q	56.3 q
$COCH_3$				20.8 q	20.8 q
$COCH_3$				168.3 s	168.2 s

100 MHz NMR (ppm): **1**, **2**, **4** and **5** in $CDCl_3$; **3** in CD_3OD.
[a~h] The assignments for these signals may be interchanged.

carried out at 28°C for 120 hours in a 300-liter fermentor with aeration at 100 liters/minute and agitation at 100 rpm (0~24 hours) and 130 rpm (24~120 hours). The production medium (200 liters) consisted of maltose syrup 5.0%, gluten meal 0.5%, soybean meal 1.0%, meat extract 0.5%, $MgSO_4 \cdot 7H_2O$ 0.1%, NaCl 0.2% and $CoCl_2 \cdot 6H_2O$ 0.001% (pH 6.5). Antibiotic activity was determined by the paper-disc method using *Bacillus subtilis* ATCC 6633 as the test organism. The fermentation broth (350 liters) from two fermentors was filtered with the aid of diatomaceous earth. The mycelial cake was extracted with a mixture of Me_2CO and water (3:2, 120 liters), and the extract was concentrated to 60 liters under reduced pressure. The antibiotics were extracted from the concentrate with EtOAc (60 liters). After removing the solvent by evaporation, the residue was washed with *n*-hexane to afford an oily material (32 g) which was applied to a silica gel (Wakogel C-200, 1.6 liters) column. After washing the column with $CHCl_3$ (3 liters), the antibiotics were eluted with a mixture of $CHCl_3$ and MeOH (50:1). The eluate was concentrated to give an oily residue (4.2 g). The residue was dissolved in MeOH (5 ml) and subjected to a column of Sephadex LH-20 (2.0 liters) eluted with MeOH. Antibiotic **2** (68 mg) was eluted first and then **1** (120 mg). Further purification by preparative-TLC (benzene - Me_2CO, 2:1) and column chromatography on Toyopearl HW-40 (MeOH, Tosoh Co.) gave **1** as an orange crystalline powder (58 mg) and **2** as an orange amorphous powder (28 mg).

Pyrindamycins A (**1**) and B (**2**) showed close similarities in the physico-chemical properties. **1**: MP 235~237°C (dec); $[\alpha]_D^{22}$ −51° (*c* 0.2,

MeOH); MS $C_{26}H_{26}N_3O_8Cl$ *m/z* 543 (M^+); UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 208 (729), 245 (sh, 360), 298 (391), 337 (580), 432 (77); IR ν_{max}^{KBr} cm^{-1} 1738 (ester), 1700 (CO), 1655 (amide), 1610. **2**: MP 173~175°C (dec); $[\alpha]_D^{22}$ −128° (*c* 0.2, MeOH); MS $C_{26}H_{26}N_3O_8Cl$ *m/z* 543 (M^+); UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 210 (991), 320 (563), 400~410 (99); IR ν_{max}^{KBr} cm^{-1} 1740 (ester), 1700 (CO), 1620 (amide). NMR spectral data for **1** and **2** are shown in Tables 1 and 2. Mild hydrolysis of **1** and **2** with 0.1 N NaOH at room temperature for 3 hours afforded 5,6,7-trimethoxyindole-2-carboxylic acid (**3**): MP 162~165°C (dec); MS $C_{12}H_{13}NO_5$ *m/z* 251 (M^+); UV λ_{max}^{MeOH} nm 214, 292. The structure of **3** was determined by its NMR spectral analysis (Tables 1 and 2).

Acetylation of **1** with acetic anhydride in pyridine afforded a monoacetate (**4**), mp 134~136°C. The high resolution (HR)-MS spectrum of **4** indicated the molecular ion peak at *m/z* 585.1492 (calcd for $C_{28}H_{28}N_3O_9Cl$: 585.1512), the fragment ion peaks attributed to **3** ($C_{12}H_{12}NO_4$, *m/z* 234.0757) and the left-half segment ($C_{16}H_{17}N_2O_5Cl$: 352.0861). The NMR spectra of **4** (Tables 1 and 2) showed the signals attributed to **3**, the left-half segment of **1** and an acetyl group at δ_H 2.38, δ_C 20.8 and 168.3. Proton decoupling experiments on the left-half segment of **4** showed the presence of partial structure $-\overset{|}{N}CH_2\overset{|}{C}H\overset{|}{C}H_2-$. By long range selective proton decoupling (LSPD) experiments on **4** it was shown that 1-H was coupled to C-2 which was further coupled to 14-H. 14-H was coupled to C-3 and C-15. Other couplings were observed as follows: 1-H to C-4, C-5 and C-15; 7-H to C-5, C-6 and C-8; and 11-H to C-8. By heteronuclear multiple-bond correlation (HMBC) experiments on **4** the long range couplings of 7-H to C-5, C-6, C-8 and C-9, and 11-H to C-8 and C-9 were observed. From the spectral analyses mentioned above the gross structure of **1** was determined. The unusual downfield shift for 7-H (8.53 ppm) in **1** may be due to the interaction with the amide carbonyl.

The stereochemistry at C-2 and C-10 of **1** was determined as *R* and *S*, respectively, by X-ray diffraction analysis. A purified preparation of **1** was crystallized from $CHCl_3$ as orange prismatic crystals. A crystal of approximate dimensions 0.2×0.2×0.05 mm was mounted on a Philips PW-1100 X-ray diffractometer. All X-ray measurements were made using graphite monochromated CuKα radiation. The lattice constants were derived from setting angles of 20 higher angle (θ=25.1°−41.7°) reflections. Crystal data: $C_{26}H_{26}N_3O_8Cl\cdot CHCl_3$, MW 663.3, orthorhombic, space group $P2_12_12_1$, a=8.266(4), b=47.403(24), c=7.690(4) Å, U=3013 Å^3, Z=4, D_{calc}=1.462 gcm^{-3}, μ for CuKα =10.2 cm^{-1}. Intensities were measured by a 2θ-ω scan method with a scan speed 0.1°/sec in ω. Backgrounds were measured at each end of the scan for half the total scan time. For weak reflections whose intensities were less than 3000 counts during the single scan, the scan were repeated once. A total of 2768 reflections in the 2θ range 6°~120° was measured. The phases of 252 strong reflections with $|E|>1.47$ were determined by MULTAN80[4] using RANTAN[5] procedure. Rotational disorder was found at the chloromethyl group of the molecule. In the final refinement, the non-hydrogen atoms were refined anisotropically by block-diagonal least-squares. The coordinate for the disordered atom was refined as though the atom was half-populated at two locations. The hydrogen atoms were eventually included in calculated positions but were not refined. The structure was refined to an R value of 0.102. The absolute configuration was determined by the anomalous dispersion method. The dispersion corrections for CuKα radiation were applied to Cl, O, N and C atoms. The calculations were done on a IBM 3090 computer using the UNICS III program[6]. An ORTEP[7] drawing of the molecule is shown in

Fig. 1. Stereoscopic view of **1** showing one of two rotational disorders.

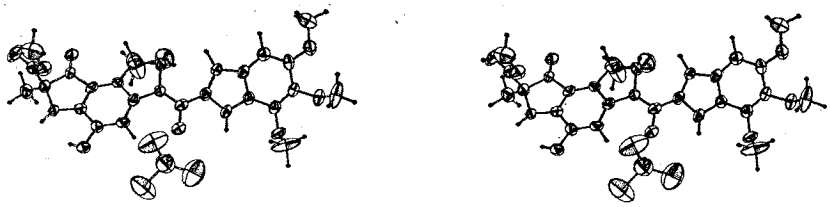


Table 3. Antimicrobial activity.

Test organisms	MIC (μg/ml)	
	1	2
Staphylococcus aureus 209P JC-1	<0.025	0.05
S. aureus Smith S-424	<0.025	<0.025
S. aureus No. 26	<0.025	<0.025
S. epidermidis ATCC 14990	0.05	0.05
S. epidermidis 109	<0.025	0.05
Enterococcus faecalis ATCC 8043	0.05	0.10
Bacillus anthracis No. 119	0.05	0.05
Escherichia coli JC-2	12.5	25
E. coli No. 29	6.25	12.5
E. coli W3630 RGN 823	6.25	12.5
Citrobacter freundii GN346	3.13	6.25
Salmonella typhi 0-901-W	3.13	3.13
S. enteritidis No. 11	0.78	1.56
S. typhimurium LT-2	12.5	25
Shigella sonnei EW 33 Type 1	3.13	6.25
Klebsiella pneumoniae PCI 602	25	50
K. pneumoniae 22 #3038	25	>100
Proteus vulgaris OX19	>100	50
P. mirabilis GN310	25	25
Providencia rettgeri J-0026	25	25
Morganella morganii Kono	50	50
Serratia marcescens MB-3848	25	25
Pseudomonas aeruginosa MB-3829	25	50
Xanthomonas maltophilia M-0627	100	100

Determined on a Sensitivity Disk-Agar medium (Nissui Seiyaku).

Fig. 1†.

Acetylation of **2** with acetic anhydride in pyridine afforded a monoacetate (**5**), mp 145~148°C, HR-MS *m/z* 585.1519, calcd for $C_{28}H_{28}N_3O_9Cl$: 585.1512. On comparison of the NMR spectra of **4** and **5**, the chemical shifts of the methine (10-H: 4.18, C-10: 42.6 ppm) of **4** were shifted to lower field (11-H: 4.50, C-11: 53.6 ppm) in **5**, but the signals of a methylene (13-H: 3.76 and 4.15, C-13: 45.8 ppm) were shifted to higher field (10-H: 3.58 and 3.71, C-10: 33.7 ppm). These results indicate the chlorine atom in **5** is attached at C-11. Consequently, these methine and methylenes make a six-membered ring in **2** and the gross structure of **2** was determined. Therefore, pyrindamycins A and B are recognized to be new antibiotics related to the naturally-occurring pyrroloindole derivatives, such as PDE I, PDE II[8] and CC-1065[1~3].

† Atomic coordinates have been deposited with the Cambridge Crystallographic Data Base and the list of Fo and Fc and other data may be obtained from YASUO TAKEUCHI upon request.

Table 4. Antitumor activity against P388 leukemia and its multiple-drug resistant cells (P388/ADR).

Dose (mg/kg)	ILS (%)		
	P388 **1**	P388 **2**	P388/ADR **2**
2.1		Toxic	Toxic
1.0		52	54
0.5	Toxic	48	41
0.25	56	44	39
0.125	48	21	
0.063	30		

Pyrindamycins A and B are strongly active against Gram-positive bacteria and active against Gram-negative bacteria, as shown in Table 3. Marked increases in life span (ILS) were observed in experiments with a single ip treatment of pyrindamycins A and B against mice ip-implanted P388 and its multiple-drug resistant (P388/ADR) leukemia cells, as shown in Table 4. Intraperitoneal LD_{50} values of pyrindamycins A and B in mice were about 0.4 and 1.0 mg/kg, respectively.

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