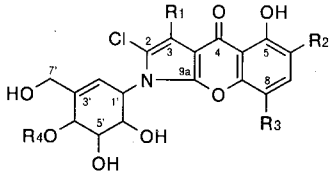
Pyralomicins, New Antibiotics from *Actinomadura spiralis*

Sir:

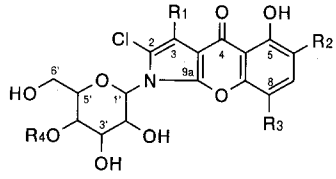
In the course of our screening program for novel antibiotics, we found that a strain of *Actinomadura spiralis* MI178-34F18 isolated from a soil sample collected on the premises of Institute of Microbial Chemistry, Tokyo, Japan, produced new antibiotics, pyralomicins (**1**~**7**, Fig. 1). In this paper we report the production, isolation, physico-chemical properties and biological properties of **1**~**7**.

A slant culture of the pyralomicins-producing organism was inoculated into a 500-ml Erlenmyer flask containing 110 ml of a seed medium consisting of galactose 2.0%, dextrin 2.0%, Bacto-soytone 1.0%, corn steep liquor 0.5%, $(NH_4)_2SO_4$ 0.2%, $CaCO_3$ 0.2% and a drop of silicon oil (adjusted to pH 7.4 before sterilization). The inoculated medium was incubated at 27°C for 72 hours on a rotary shaker. This pre-seed culture was transferred to two 30-liter jar fermenters each containing 12 liters of the same medium above described.

Fig. 1. The structures of pyralomicins 1a, 1b, 1c, 1d, 2a, 2b and 2c.



	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

Fig. 2. Isolation and purification procedure for pyralomicins 1a, 1b, 1c, 1d, 2a, 2b and 2c.

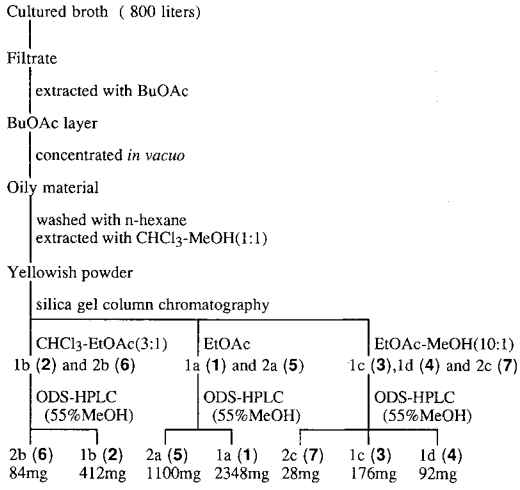


Table 1. Physico-chemical properties of pyralomicins 1a, 1b, 1c, 1d, 2a, 2b and 2c.

	1a (**1**)	1b (**2**)	1c (**3**)	1d (**4**)	2a (**5**)	2b (**6**)	2c (**7**)
Appearance	Pale yellow powder	Pale yellow powder	Pale yellow powder	Pale yellow powder	Pale yellow powder	Pale yellow powder	Pale yellow powder
MP	297-300 °C (dec)	254-257 °C (dec)	277-280 °C (dec)	298-300 °C (dec)	295-297 °C (dec)	263-267 °C (dec)	266-269°C (dec)
$[\alpha]_D^{23}$	-118.8° (*c* 0.5, DMF)	-102.0° (*c* 0.5, DMF)	-143.6° (*c* 0.5, DMF)	-133.9° (*c* 0.33, DMF)	+13.2° (*c* 0.25, DMF)	+24.4° (*c* 0.6, DMF)	+2.8° (*c* 0.5, DMF)
Molecular formula	$C_{20}H_{19}NO_7Cl_2$	$C_{20}H_{19}NO_7Cl_2$	$C_{19}H_{17}NO_7Cl_2$	$C_{19}H_{16}NO_7Cl_3$	$C_{19}H_{19}NO_8Cl_2$	$C_{19}H_{19}NO_8Cl_2$	$C_{18}H_{17}NO_8Cl_2$
HRFAB-MS (m/z)							
Calcd:	456.0617	456.0617	442.0460	476.0071	460.0566	460.0566	446.0409
Found:	456.0611	456.0628	442.0466	476.0054	460.0554	460.0582	446.0405
UV λ_{max} nm,(log ε) in							
MeOH	249 (4.45), 290 (sh,3.70), 355 (4.00)	246 (4.50), 285 (sh,3.74), 357 (4.07)	249 (4.49), 285 (sh,3.75), 356 (4.03)	251 (4.45), 280 (sh,3.78), 357 (3.92)	248 (4.56), 290 (sh,3.65), 354 (4.03)	246 (4.56), 285 (sh,3.66), 356 (4.04)	248 (4.56), 290 (sh,3.70), 354 (3.99)
0.1N NaOH-90% MeOH	235 (sh,4.38), 254 (4.34), 290 (sh,3.76), 390 (4.05)	232 (sh,4.50), 248 (4.47), 285 (sh,3.86), 382 (4.00)	232 (sh,4.49), 254 (4.45), 283 (sh,3.86), 390 (4.08)	233 (sh,4.41), 254 (4.35), 290 (sh,3.69), 389 (4.02)	235 (sh,4.42), 253 (4.42), 294 (sh,3.78), 391 (4.09)	237 (sh,4.50), 246 (4.50), 287 (sh,3.79), 390 (3.90)	234 (sh,4.49), 251 (4.44), 285 (sh,3.88), 391 (4.08)
IR ν max(KBr) cm^{-1}	3400, 2925, 1645, 1600, 1590(sh), 1535, 1520, 1460, 1405, 1320, 1280, 1265(sh), 1205, 1110, 740	3390, 2920, 1630, 1610, 1525, 1510(sh), 1460, 1440, 1410, 1380, 1305, 1270, 1220, 1195, 1100, 1075, 1030, 790, 755	3385, 2930, 1645, 1600, 1590, 1530, 1515, 1455, 1405, 1315, 1275, 1265, 1205, 1090, 990, 740	3400, 2920, 1640, 1600, 1545, 1525, 1450, 1400, 1310, 1270, 1200, 1185, 1105, 1090(sh), 990, 755	3390, 2920, 1640, 1595, 1530(sh), 1515, 1450, 1400, 1320, 1285, 1265, 1210 1125, 1090, 1060, 740	3390, 2920, 1630, 1610, 1530(sh), 1515, 1460, 1440, 1405, 1380, 1305, 1280, 1265, 1210 1120, 1090, 1075, 1060(sh), 780, 760	3370, 2930, 1645, 1600, 1585, 1530(sh), 1520, 1455, 1405, 1325, 1290, 1270, 1210, 1085, 1060, 780, 740
HPLC Rt * min	22.7	31.2	18.5	28.4	17.8	24.2	13.8

* mobile phase : 55%MeOH aq., flow rate: 0.7ml/min, (Senshu Pak, Nucleosil $5C_{18}$ 4.6φ × 250mm)

The fermentation was carried out at 27°C for 96 hours. This seed culture was transferred to a 2,000-liter tank fermenter containing 800 liters of a production medium consisting of starch 3.0%, corn steep liquor 0.5%, yeast extract 0.2%, $MgSO_4 \cdot 7H_2O$ 0.05%, NaCl 0.3%, $CoCl_2 \cdot 6H_2O$ 0.001%, $CaCO_3$ 0.3% and a small amount of silicone oil (adjusted to pH 7.2 before sterilization). The fermentation was carried out at 28°C under aeration of 400 liters/minute and agitation of 70 rpm for 114 hours.

The isolation procedure of pyralomicins is illustrated in Fig. 2. This procedure was monitored by antimicrobial assay against *Micrococcus luteus* IMC B-0026 (IFO 3333).

Physico-chemical properties of pyralomicins are summarized in Table 1. Pyralomicins are soluble in dimethylsulfoxide, dimethylformamide, slightly soluble in methanol, isopropanol and insoluble in *n*-hexane and

Table 2. ^1H NMR assignments of pyralomicins 1a, 1b, 1c, 1d, 2a, 2b and 2c in DMF-d_7.

Position	1a (1)	1b (2)	1c (3)	1d (4)	2a (5)	2b (6)	2c (7)
3	6.74 (s)	6.74 (s)	6.74 (s)	-	6.80 (s)	6.80 (s)	6.79 (s)
5-OH	13.81 (brs)	13.47 (brs)	13.82 (brs)	13.60 (brs)	13.68 (brs)	13.40 (brs)	13.69 (brs)
6-CH_3	-	2.25 (s)	-		-	2.27 (s)	-
7	7.71 (s)	7.72 (s)	7.71 (s)	7.73 (s)	7.76 (s)	7.77 (s)	7.75 (s)
8-CH_3	2.38 (s)	-	2.36 (s)	2.34 (s)	2.48 (s)	-	2.46 (s)
1'	5.15 (brd)	5.15 (brd)	5.17 (brd)	5.17 (brd)	5.53 (d, 9.3)	5.49 (d, 9.3)	5.52 (d, 9.3)
2'	5.82 (brd)	5.74 (brs)	5.74 (brd)	5.80 (brd)	4.39 (dt, 5.4, 9.3)	4.55 (dt, 5.4, 9.3)	4.37 (brt)
2'-OH	-	-	-	-	5.91 (d, 5.4)	5.90 (d, 5.4)	5.88 (brs)
3'	-	-	-	-	3.77 (m)	3.77 (dt, 4.4, 9.3)	3.63 (m)
3'-OH	-	-	-	-	5.62 (d, 4.9)	5.64 (d, 4.4)	5.33 (brs)*
4'	4.13 (brd)	4.16 (brd)	4.24 (brd)	4.35 (brd)	3.44 (t, 9.3)	3.35 (t, 9.3)	3.63 (m)
4'-OH	-	-	5.35 (d, 5.4)	5.37 (brd)	-	-	5.50 (brs)*
4'-OCH_3	3.60 (s)	3.59 (s)	-	-	3.63 (s)	3.61 (s)	-
5'	3.89	3.88 (m)	3.72	3.72 (brt)	3.65 (m)	3.65 (m)	3.63 (m)
	(ddd, 4.4, 7.8, 9.8)		(ddd, 3.4, 7.8, 10.3)				
5'-OH	5.40 (d, 4.4)	5.43 (brs)	5.31 (d, 3.4)	5.33 (brs)	-	-	-
6'	4.28 (m)	4.38 (m)	4.37 (m)	4.20 (m)	3.73 (m), 3.83 (m)	3.69 (m), 3.84 (m)	3.74 (m), 3.90 (m)
6'-OH	5.72 (d, 4.4)	5.72 (brd)	5.61 (brd)	5.68 (brs)	4.93 (t, 5.9)	4.80 (brt)	4.81 (brt)
7'	4.22 (m)	4.22 (brs)	4.29 (m)	4.29 (m)	-	-	-
7'-OH	5.01 (brt)	4.87 (brt)	4.93 (brt)	4.95 (br)	-	-	-

Chemical shifts in ppm from TMS as an internal standard.
Multiplicity and *J* value (Hz) are in parentheses.
* These signals are exchangeable.

Table 3. ^{13}C NMR assignments of pyralomicins 1a, 1b, 1c, 1d, 2a, 2b and 2c in DMF-d_7.

Position	1a (1)	1b (2)	1c (3)	1d (4)	2a (5)	2b (6)	2c (7)
2	119.8	119.5	119.8	117.2*	119.1	119.0	119.1
3	99.7	99.9	99.7	102.6*	100.6	100.8	100.5
3a	105.2	105.3	105.2	103.6*	105.5	105.6	105.5
4	177.5	177.6	177.5	177.2	177.7	177.8	177.7
4a	110.1	109.8	110.1	110.1	110.1	109.9	110.1
5	155.5	158.9	155.5	155.5	155.4	158.8	155.4
6	114.2	122.1	114.3	114.5	114.3	122.2	114.3
6-CH_3	-	14.4	-	-	-	14.4	-
7	135.6	135.8	135.9	136.1	135.9	136.0	135.9
8	117.8	109.8	117.9	117.9	118.0	109.9	117.9
8-CH_3	14.8	-	14.7	14.6	14.9	-	14.8
8a	151.4	148.1	151.4	151.2	151.5	148.3	151.5
9a	150.2	149.8	150.2	148.8	150.4	149.9	150.4
1'	60.7	61.0	61.2	62.3	86.0	86.0	86.3
2'	119.4	118.3	117.3	117.0	71.9	71.5	71.8
3'	143.5	144.0	145.1	145.2	77.9	78.0	78.0
4'	82.8	82.9	73.7	73.7	79.8	80.2	70.9
4'-OCH_3	59.4	59.4	-	-	60.5	60.5	-
5'	76.8	76.7	78.0	77.8	79.6	79.9	81.0
6'	73.0	72.8	72.9	73.1	61.4	62.0	62.0
7'	61.8	61.7	61.9	61.9	-	-	-

Chemical shifts in ppm from TMS as an internal standard.
* These signals are exchangeable.

Table 4. The antimicrobial activities of pyralomicins 1a, 1b, 1c, 1d, 2a, 2b and 2c.

Test organisms	MIC (μg/ml)						
	1a (**1**)	1b (**2**)	1c (**3**)	1d (**4**)	2a (**5**)	2b (**6**)	2c (**7**)
Staphylococcus aureus FDA209P	>50	50	>50	>50	50	>50	>50
S.aureus Smith	>100	50	100	>100	50	100	>100
Micrococcus luteus FDA16	0.78	0.39	0.20	25	0.78	12.5	1.56
M. luteus IFO3333	3.12	0.39	0.20	25	0.78	6.25	3.12
Bacillus anthracis	>50	25	>50	>50	25	50	>50
B. subtilis NRRL B-558	>100	100	>100	>100	25	100	>100
B. cereus ATCC 10702	>100	25	100	100	25	50	100
Corynebacterium bovis 1810	>50	50	6.25	>50	25	50	>50
Escherichia coli NIHJ	>100	100	100	>100	100	>100	100
E. coli BE1121	100	1.56	1.56	3.12	1.56	6.25	1.56
E. coli BE1186	>50	12.5	6.25	50	12.5	25	25
Shigella dysenteriae JS11910	>50	50	12.5	>50	50	>50	25
Candida albicans 3147	>50	>50	>50	>50	>50	>50	>50

Mueller Hinton agar (Difco) 37°C, 18 hrs

water. The molecular formulae for pyralomicins were determined by HRFAB-MS.

The structures of pyralomicins were determined as shown in Fig. 1 by various NMR spectral analyses including ^{1}H-^{15}N HMBC[1,2] improved with the decoupled-HMBC (D-HMBC) technique[3], and $^{13}C\{^{1}H\}$ NOE[4] experiments.

The ^{1}H and ^{13}C NMR data of pyralomicins are shown in Tables 2 and 3. Pyralomicins were determined to be a new type of antibiotics. Pyralomicins 1a~1d (**1**~**4**) and pyralomicins 2a~2c (**5**~**7**) have a benzopyranopyrrole ring[5] as a chromophore. Pyralomicins 1a~1d (**1**~**4**) have a cyclohexene ring connected to the chromophore through a nitrogen atom. Another series of pyralomicins, pyralomicins 2a~2c (**5**~**7**) have a tetrahydropyran ring in place of the cyclohexene ring. Details of the structure determination of pyralomicins will be reported later.

The antimicrobial activities of pyralomicins are shown in Table 4. Pyralomicins inhibited the growth of *Micrococcus luteus* IMC B-0026 (IFO3333) at the concentration of 0.2~25 μg/ml by agar dilution method. Pyralomicin 1a (**1**) and pyralomicin 2a (**5**) did not show any acute toxicity in mice at 100 mg/kg ip.

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