KIJIMICIN, A POLYETHER ANTIBIOTIC

Sir:

A new polyether antibiotic, kijimicin was found in culture filtrate of an *Actinomadura* sp. MI215-NF3, which was isolated from a soil sample collected at Bunkyo-ku, Tokyo, Japan. The antibiotic showed higher anticoccidial activity than monensin or salinomycin and further studies are now in progress. In this communication, the production, isolation, physico-chemical properties, structure and biological properties are reported.

A slant culture of the producing organism was inoculated into a 500-ml Erlenmeyer flask containing 110 ml of a seed medium consisting of galactose 2.0%, dextrin 2.0%, Bacto-Soytone (Difco) 1.0%, corn steep liquor 0.5%, $(NH_4)_2SO_4$ 0.2%, $CaCO_3$ 0.2% (adjusted to pH 7.4 before sterilization). After incubation at 30°C for 6 days on a rotary shaker, 3.5 ml portion of this culture was transferred to 110 ml of a production medium consisting of glycerol 1.5%, soluble starch 1.5%, Soybean meal (Ajinomoto) 0.5%, fish meal 1.5%, $CaCO_3$ 0.2% (adjusted to pH 7.4 before sterilization). The fermentation was carried out at 27°C for 6 days on a rotary shaker. Kijimicin was purified using Sephadex LH-20 and centrifugal partition chromatography (Sanki Engineering Limited, model NMF) and finally crystallized from ethyl acetate - hexane (Fig. 1).

Physico-chemical properties of kijimicin Na-salt are summarized in Table 1. Chemical shifts in the ^{13}C and ^{1}H NMR spectra of the antibiotic are shown in Table 2. The structure determination of kijimicin was carried out by NMR spectroscopic analyses: DEPT spectrum, ^{1}H-^{1}H COSY and heteronuclear multiple-bond connectivity (HMBC) spectrum as the structures of portmicin[1] and hidamicin[2] were determined by these techniques. Thus, the planar structure of the antibiotic was proposed as shown in Fig. 2, though the connectivities of C-16 ~ C-17 and C-20 ~ C-21 were not determined definitively.

To confirm these connectivities and to determine the absolute structure, the X-ray crystallographic study of kijimicin Rb-salt was carried out. The lattice parameters and intensity data were measured on a Philips PW1100 diffractometer using graphite-monochromated CuKα radiation. The crystal data are: Kijimicin Rb-salt hemi-hexane solvate, $C_{37}H_{63}O_{11}Rb \cdot \frac{1}{2}(C_6H_{14})$, FW = 812.5. Orthorhombic, space group $P2_12_12_1$, $Z=4$, $a=18.799(10)$, $b=19.798(10)$, $c=12.789(7)$ Å, $U=4759.8$ Å^3. $D_{calc}=1.134$ gcm^{-3}, μ for CuKα = 18.26 cm^{-1}.

Of the 4,566 theoretically possible reflections within the 2θ range of 6° ~ 140°, 2,799 reflections were measured as above the 2σ (I) level. A total of 520 Friedel pairs in the 2θ range 20° ~ 60° were measured. The structure was determined by the heavy atom method and refined by the block-diagonal least-squares calculations. All the

Fig. 1. Isolation and purification of kijimicin.

Culture filtrate (15.0 liters)
↓ extracted with EtOAc (3 liters × 4) at pH 8.0
Organic layer
↓ dried over Na_2SO_4, evaporated *in vacuo*
Brown oil (2.2 g)
↓ dissolved in EtOAc (100 ml), washed with 0.1 N HCl (30 ml × 3), washed with 0.1 N NaOH (30 ml × 3), washed with satd NaCl (30 ml × 3)
Organic layer
↓ dried over Na_2SO_4, evaporated *in vacuo*
Brown oil (1.1 g)
↓
Sephadex LH-20 column chromatography (550 ml)
↓ eluted with acetone - hexane (1 : 1)
Active fraction
↓ evaporated *in vacuo*
Pale yellow oil (1.0 g)
↓
Centrifugal partition chromatography
↓ solvent system: acetonitrile - hexane
Active fraction
↓ evaporated *in vacuo*, crystallized from EtOAc - hexane
Pure kijimicin Na-salt (colorless plate: 750 mg)

Table 1. Physico-chemical properties of kijimicin Na-salt.

SI-MS (*m/z*)	707 (MH^+)
Analysis	
Calcd for $C_{37}H_{63}O_{11}Na$:	C 62.87, H 8.98, O 24.90, Na 3.25
Found:	C 62.47, H 8.96, O 24.60, Na 3.53
$[\alpha]_D^{25}$ (*c* 1.0, $CHCl_3$)	+30.6°
MP	217 ~ 218°C
IR(KBr) cm^{-1}	3430, 2980, 1585, 1465, 1410, 1380, 1365, 1320, 1185, 1110, 1070, 990, 945, 880, 700

Table 2. ^{13}C and ^{1}H NMR chemical shifts of kijimicin Na-salt in $CDCl_3$.

Position	δ_C (ppm)	δ_H (ppm)	Assignment	Position	δ_C (ppm)	δ_H (ppm)	Assignment
1	182.86		COONa	20	77.94	4.26 (dd, 6.6, 9.6)	CH(O)
2	44.00	2.61 (dq, 6.6, 10.0)[a]	CH	21	106.14		C(O) (O)
3	82.32	3.23 (dd, 10.0, 2.6)	CH(O)	22	38.98	1.89	CH
4	35.98	2.13	CH	23	35.37	1.50, 1.94	CH_2
5	66.58	4.04 (dd, 11.6, 1.8)	CH(O)	24	86.47		C(O)
6	30.95	2.31	CH	25	74.58	3.42 (dd, 10.4, 2.2)	CH(O)
7	87.20	2.97 (dd, 2.4, 4.4)	CH(O)	26	26.09	1.08, 1.36	CH_2
8	33.83	2.01	CH	27	10.79	0.95 (t, 7.6)	CH_3
9	108.81		C(O) (O)	28	24.97	1.13 (s)	CH_3
10	33.11	1.68, 1.78	CH_2	29	13.43	0.96 (d, 7.2)	CH_3
11	24.89	1.37, 2.17	CH_2	30	15.65	0.89 (d, 8.0)	CH_3
12	75.85	4.49 (ddd, 7.8, 4.8, 2.6)	CH(O)	31	20.90	1.11 (s)	CH_3
13	76.26	3.93 (dt, 8.6, 2.6)	CH(O)	32	12.03	0.98 (d, 7.0)	CH_3
14	22.80	1.78, 1.85	CH_2	33	10.65	0.92 (d, 7.6)	CH_3
15	29.06	1.46, 2.05	CH_2	34	10.17	1.03 (d, 6.6)	CH_3
16	84.42		C(O)	35	16.40	1.19 (d, 6.6)	CH_3
17	84.50	4.22 (d, 4.4)	CH(O)	36	60.34	3.49 (s)	OCH_3
18	35.07	2.31	CH	37	57.68	3.35 (s)	OCH_3
19	35.42	1.58, 2.16	CH_2				

[a] Proton signal multiplicity and coupling constant (J=Hz).

Fig. 2. Structure of kijimicin.

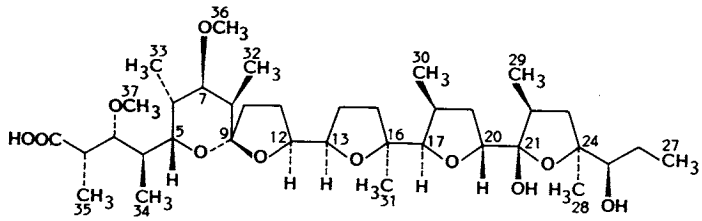
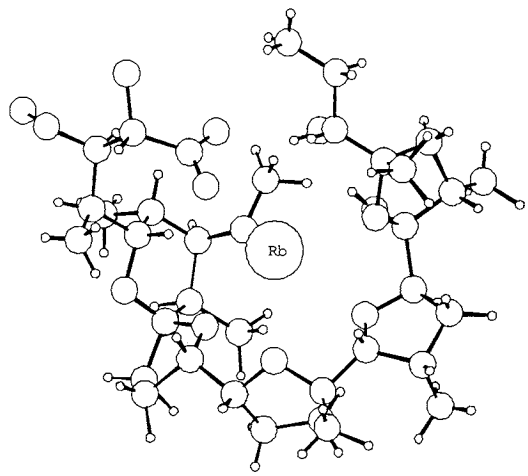


Fig. 3. Molecular structure of kijimicin Rb-salt.



heavier atoms were located on the difference electron-density map and refined to an R-value of 0.134. Absolute configuration was determined by the anomalous dispersion method. Of 105 Friedel pairs for which both the calculated and observed ratios of $|F(hkl)|/|F(\bar{h}\bar{k}\bar{l})|$ differ more than 3% from the unity, all pairs showed consistently the absolute configuration of the molecule as shown in Fig. 3. Finally, a difference electron-density map was calculated which showed diffuse and elongated peaks assignable to two solvent hexane molecules. The final least-squares calculations including kijimicin molecule, hexane C atoms (with multiplicity factors of 0.25) and 57 hydrogen atoms gave the R-value of 0.105†.

Among known polyether antibiotics, kijimicin most resembles portmicin but lacks a sugar at C-14. The antimicrobial activities of kijimicin were measured by an agar dilution method using Mueller-Hinton agar. The antibiotic inhibited mainly the growth of Gram-positive bacteria (Table

† The atomic parameters, bond lengths and angles have been sent to the Cambridge Crystallographic Centre.

Table 3. The antimicrobial activities of kijimicin.

Test organism	MIC (μg/ml)	Test organism	MIC (μg/ml)
Staphylococcus aureus FDA 209P	<0.78	*Corynebacterium bovis* 1810	<0.78
S. aureus Smith	1.56	*Mycobacterium smegmatis* ATCC 607	12.5
S. aureus MS9610	<0.78	*Escherichia coli* NIHJ	>100
Micrococcus luteus FDA 16	1.56	*E. coli* BEM 11	>100
M. luteus IFO 3333	3.12	*E. coli* BE 1121	3.12
M. luteus PCI 1001	<0.78	*E. coli* BE 1186	3.12
Bacillus anthracis	0.78	*Shigella dysenteriae* JS 11910	25
B. subtilis NRRL B-558	1.56	*Salmonella typhi* T-63	>100
B. subtilis PCI 219	1.56	*S. enteritidis* 1891	100
B. cereus ATCC 10702	<0.78	*Klebsiella pneumoniae* PCI 602	100

Table 4. Anticoccidial activities of kijimicin, monensin and salinomycin.

Compound	Dose (ppm)	Mean weight gain (%)	Survival rate (%)	Oocyst scores	Lesion scores	A.C.I.[a]
Kijimicin Na-salt	50	92.23	100	10	26.0	156.8
Monensin Na-salt	100	95.52	100	20	32.0	144.1
Salinomycin Na-salt	50	70.68	100	10	34.0	127.1
None	0	82.42	100	20	40.0	122.9

[a] Anticoccidial index (A.C.I.) = (mean weight gain + survival rate) − (oocyst scores + lesion scores).

3). The antibiotic, monensin and salinomycin were tested for experimental chicken coccidiosis infected with *Eimeria tenella* (Table 4). By this preliminary test, kijimicin was superior to monensin or salinomycin. The acute toxicities (LD_{50}) of the antibiotic in mice are 56 mg/kg (ip) and 180 mg/kg (po).

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