SANDRAMYCIN, A NOVEL ANTITUMOR ANTIBIOTIC PRODUCED BY A *NOCARDIOIDES* SP.

PRODUCTION, ISOLATION, CHARACTERIZATION AND BIOLOGICAL PROPERTIES

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A new antitumor antibiotic, sandramycin, was isolated from cultured broth of a *Nocardioides* sp. (ATCC 39419) and purified by solvent partition and column chromatography. Sandramycin, a new depsipeptide, was moderately active *in vitro* against Gram-positive organisms and *in vivo* against leukemia P388 in mice.

In the course of screening for new antitumor agents from culture broths, we discovered a new antitumor antibiotic compound, sandramycin, produced by a *Nocardioides* sp. (ATCC 39419). Sandramycin is closely related to the cyclic depsipeptides luzopeptins A, B, C and D[1~3]. This paper details the production, isolation, physico-chemical and biological properties of sandramycin. Details of the structure determination will be reported elsewhere[4].

Fermentation

The producing organism *Nocardioides* sp. (ATCC 39419) was isolated from a soil sample collected in Mexico. It was stored and maintained on yeast extract-malt extract, agar slants consisting of glucose 0.4%, yeast extract 0.4%, malt extract 1% and agar 2%, pH 7.0. A vegetative inoculum for shake flask or submerged fermentations was prepared by transferring the mycelial growth from a slant culture to 100 ml of vegetative medium consisting of glucose 30%, soy flour 10%, cottonseed embryo meal 10% and $CaCO_3$ 3% in a 500-ml Erlenmeyer flask. The flask was incubated at 27°C for 48 hours on a rotary shaker (210 rpm).

For shake flask fermentations, 4 ml of vegetative inoculum was added to 100 ml of sterile production media in a 500-ml Erlenmeyer flask. The production media consisted of sucrose 2%, soy flour 1%, linseed meal 1% and $CaCO_3$ 0.5%. The flasks were incubated at 27°C on a rotary shaker (250 rpm). The fermentation was harvested for extraction at 96 hours.

For submerged fermentations, a production media of corn starch 4%, linseed meal 2%, $(NH_4)_2SO_4$ 0.1% and $CaCO_3$ 0.5% was used. For bench top fermenters, 400 ml of vegetative inoculum was added to 10 liters of production media. The temperature within the tank was maintained at 27°C with an agitation rate of 300 rpm and air flow of 8 liters/minute. The fermentation was harvested for extraction at 202 hours. For tank fermentation, 2 liters of vegetative inoculum was added to 30 liters of production media. The temperature within the tank was maintained at 26.5°C with an agitation rate of 375 rpm and an air flow of 80 liters/minute. After 183 hours, the fermentation was harvested for extraction.

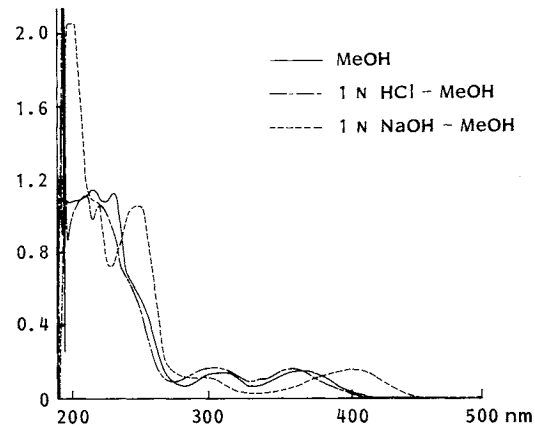
Isolation and Purification

A crude preparation of sandramycin was isolated from the fermentation broth by solvent extraction. Whole broth (8 liters) was extracted with ethyl acetate (8 liters) and filtered with filter aid. The mycelial cake was washed with 2 liters of ethyl acetate. The extract was evaporated to dryness *in vacuo* to yield 1.94 g of crude solid.

The crude solid was partitioned between hexanes (3 × 200 ml) and 90% methanol (22 ml water - 200 ml methanol). The methanol layer was diluted (44 ml water) to 75% methanol and partitioned against pre-equilibrated carbon tetrachloride (3 × 200 ml). The methanol layer was diluted (42 ml water) to 65% methanol and partitioned against pre-equilibrated chloroform (3 × 200 ml). The residues obtained from the carbon tetrachloride fraction (595 mg) and chloroform fraction (458 mg) were combined.

The composite was dissolved in 30 ml of chloroform - methanol (2 : 1) and mixed with 17.4 g of diatomaceous earth. The solvents were evaporated *in vacuo* in a rotatory evaporator. The resulting powder was packed as a hexane slurry (500 ml) into a flash chromatography column with a pressurized flow (N_2, 0.4 kg/cm^2). Elution continued under pressure with 1 liter of toluene. The toluene eluant was evaporated *in vacuo* to yield 0.699 g of residue. This residue was dissolved in chloroform and charged onto a column (94 ml) of silica gel (37 g). The column was eluted with a 2-liter linear gradient of chloroform to 5% methanol in chloroform collecting 20 × 100 ml fractions. The residue from each fraction was assayed by silica gel TLC (methanol - chloroform, 95 : 5). Fraction 6 was judged homogeneous and crystallized from chloroform - methanol to yield 215 mg of sandramycin.

Fig. 1. UV spectrum of sandramycin.



Physico-chemical Properties

Sandramycin was obtained as a white solid from chloroform - methanol crystallization: MP 208~212°C. The crystals would gradually yellow with prolong exposure to laboratory light but no

Table 1. Physico-chemical properties of sandramycin.

Appearance	White powder
MP	208~212°C
Molecular formula	$C_{60}H_{76}O_{16}N_{12}$
FAB-MS	
Calcd:	1,221.5579 $(M+H)^+$
Found:	1,221.5571 $(M+H)^+$
Analysis	$C_{60}H_{76}O_{16}N_{12}\cdot 8H_2O$
Calcd:	C 52.78, H 6.79, N 12.31
Found:	C 52.58, H 6.27, N 12.29
UV λ_{max}^{MeOH} nm (*a*)	217 (63.7), 229 (62.8), 356 (8.1)
$\lambda_{max}^{MeOH-HCl}$ nm (*a*)	210 (62.3), 228 (58.4), 306 (8.1), 356 (8.3)
$\lambda_{max}^{MeOH-NaOH}$ nm (*a*)	246 (59.8), 301 (7.2), 395 (9.2)
IR (KBr) cm^{-1}	3500, 3340, 1748, 1660, 1640, 1520

FAB: Fast atom bombardment.

Fig. 2. IR spectrum of sandramycin (KBr).

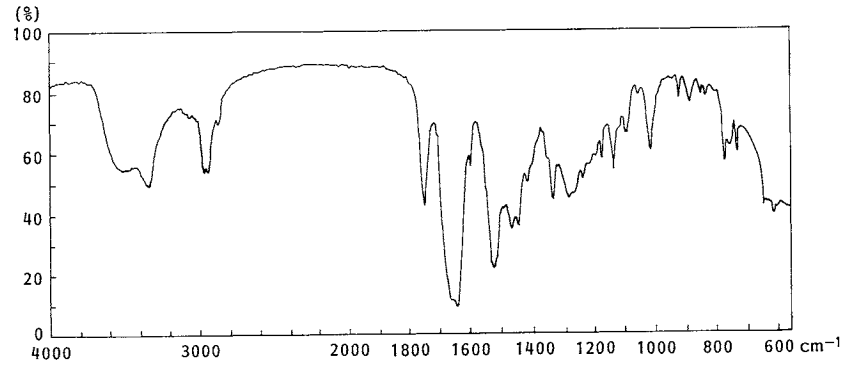
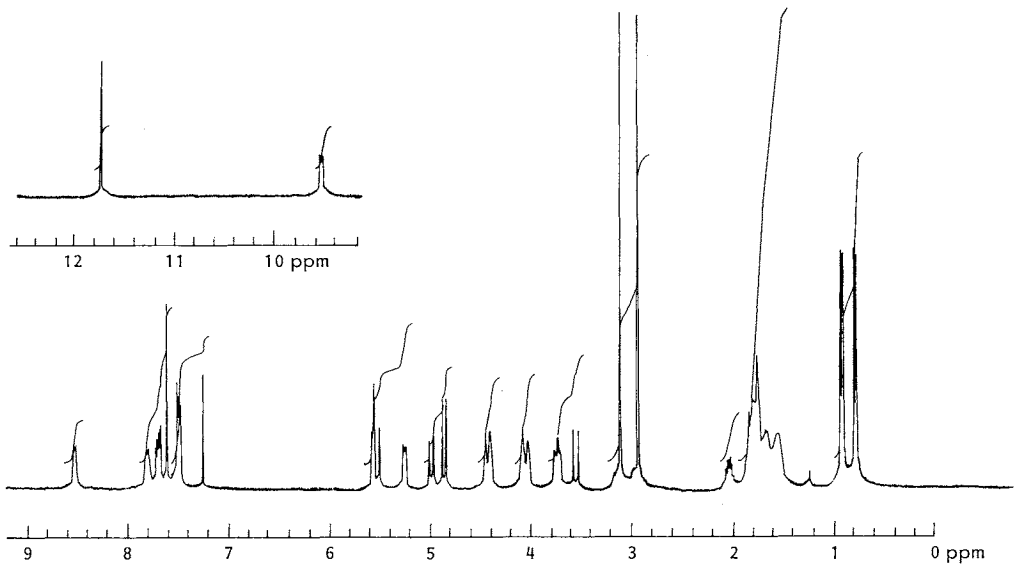


Fig. 3. ^1H NMR spectrum of sandramycin in $CDCl_3$.



degradation product was detected spectroscopically. The compound was soluble in chloroform, methylene chloride, and ethyl acetate, slightly soluble in toluene and methanol, and insoluble in hexanes and water. It had an Rf of 0.4 on silica gel using chloroform - methanol (95 : 5) as eluant and iodine vapor or fluorescence with 366 nm UV excitation for detection. The molecular formula of $C_{60}H_{76}O_{16}N_{12}$ was established by elemental analysis and high resolution MS. Other physicochemical properties of sandramycin are listed in Table 1. The UV, IR and ^1H NMR spectra of sandramycin are shown in Figs. 1, 2 and 3,

Fig. 4. Structure of sandramycin.

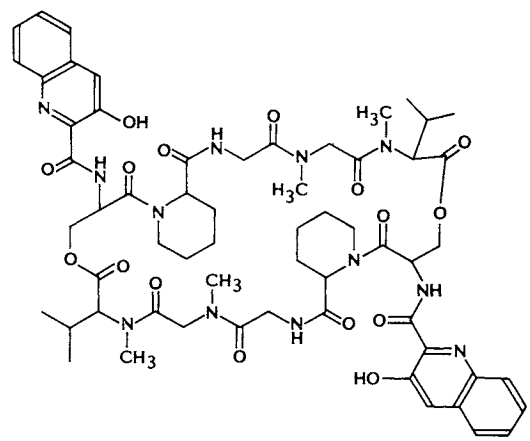


Table 2. Antimicrobial activity of sandramycin.

Test organism	MIC (μg/ml)		
	Sandramycin	Luzopeptin A	Echinomycin
Bacillus subtilis (Rec$^+$) A22508-2	0.024	0.195	0.049
B. subtilis (Rec$^-$) A22509-2-2	0.012	0.049	<0.003
Staphylocccus aureus 209P-A9497	0.012	0.098	0.012
S. aureus (echinomycin-resistant) A9628	0.098	0.098	0.78
Streptococcus faecalis A9611	0.024	0.195	0.012
Escherichia coli A15119	12.5	12.5	12.5
E. coli (actinomycin-sensitive) A21780 (AS-19)	12.5	12.5	6.25

Rec: Recombinational repair gene *rec*A.

Table 3. Antitumor activity of sandramycin against leukemia P388.

Compound	Treatment schedule	Dose (ip, mg/kg/injection)	MST (days)	T/C (%)	AWC (g, day-4)	Survivors (day-5)
Sandramycin	d 1	3.2	Toxic	Toxic	−2.8	0/6
		1.6	11.5	128	−1.3	6/6
		0.8	10.0	111	−1.6	6/6
		0.4	11.0	122	−0.7	6/6
		0.2	14.5	161	−1.4	6/6
		0.1	11.0	122	−0.9	6/6
		0.05	10.0	111	−0.6	6/6
		0.025	9.5	106	−0.2	6/6
Sandramycin	d 1→5	1.6	Toxic	Toxic	−1.7	2/6
		0.8	6.0	67	−1.4	5/6
		0.4	11.0	122	−1.3	6/6
		0.2	12.0	133	−1.7	5/6
		0.1	11.5	128	−0.8	6/6
		0.05	8.5	94	−1.8	6/6
		0.025	10.0	111	−1.4	5/5
		0.0125	10.5	117	−0.9	6/6
Control		Saline	9.0	—	0.2	10/10

Tumor inoculum: 10^6 ascites cells implanted ip. Host: CDF_1 female mice. Evaluation: Median survival time (MST). Effect: T/C (%)≥(MST treated/MST control)×100. Criteria: T/C (%)=125 considered significant antitumor activity.

respectively. The details of the chemical structure (Fig. 4) determination will be reported elsewhere[4].

Biological Activities of Sandramycin

Antimicrobial activities of sandramycin were determined by a serial 2-fold dilution method. The results are shown in Table 2 in comparison with luzopeptin A and echinomycin. Sandramycin was strongly inhibitory to the Gram-positive organisms *Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus faecalis*. No induction of lysogenic bacteria (ILB test) was observed.

Antitumor activity was measured *in vivo* against transplantable mouse leukemia P388. The results are shown in Table 3. Sandramycin was moderately active in the P388 tumor model with values comparable to those observed for luzopeptin A (BBM-928)[5].

Conclusion

Sandramycin is a new cyclic depsipeptide antitumor antibiotic. Its antimicrobial activity parallels

that which is observed for echinomycin and luzopeptin A. Unformulated compound yielded erratic antitumor data against leukemia P388 in mice. The best increase in life span over the controls was 61% (T/C % 161%) at 0.2 mg/kg/injection using single dose treatment. The compound will be evaluate against other *in vivo* murine tumor models.

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