Communications to the editor

ANTLERMICINS B AND C, NEW MEMBERS OF THE ANTLERMICIN FAMILY

Sir:

We report here isolation and characterization of two members of antitumor antibiotics antlermicins, which are produced by *Micromonospora chalcea* subsp. *kazunoensis*.

Fermentation of the strain is the same as described before[1]. The ethyl acetate extracts from the culture filtrate and mycelium were subjected to the successive silicic acid chromatographies using the following solvent systems: (1) Benzene - methanol (10: 1), (2) ethyl acetate - acetone (7: 1→5: 1). Most of antlermicin A was eluted with ethyl acetate - acetone (7: 1). Fractions eluted with ethyl acetate - acetone (5: 1) (4 g from 360 liters of culture broth) contains two additional active components besides antlermicin A. After purification by a Diaion HP-20 column (60% acetone→acetone), they were separated by preparative thin-layer chromatography using Merck Silica gel 60 F-254 plates with the solvent system, chloroform - methanol (5: 1). Rf values were as follows: antlermicin A 0.54, B 0.51, C 0.44. Fractions of antlermicins B and C were dissolved in ethyl acetate and washed with 0.1 N hydrochloric acid, then with water. Each organic layer was dehydrated with sodium sulfate, concentrated *in vacuo*, and finally precipitated by adding hexane, affording white powder of free acids of antlermicins B (80 mg) and C (70 mg).

Physico-chemical properties of antlermicins B and C are summarized in Table 1. Both compounds are positive to potassium permanganate, periodate-benzidine, chlorine-tolidine, 2,4-dinitrophenylhydrazine, and *o*-dianisidine tests. They give olive-blue color with sulfuric acid, dark brown color with anthrone reagent and blue color with ELSON-MORGAN test. The UV, IR and ^{13}C NMR spectra are shown in Figs. 1, 2, and 3 respectively.

Antimicrobial spectra of antlermicins B and C are shown in Table 2. Like antlermicin A, they are most active to Gram-positive bacilli and less active to Gram-positive cocci. They showed no activity against Gram-negative bacteria, mycobacteria, yeasts, and fungi tested. They are

Table 1. Physico-chemical properties of antlermicins B and C.

	Antlermicin B	Antlermicin C
Melting point	210~214°C	229~236°C
Elementary analysis	C 60.60%	C 60.71%
	H 7.24	H 7.16
	N 2.18	H 2.77
Empirical formula	$C_{61\sim66}H_{86\sim96}N_2O_{22\sim24}$	$C_{46\sim50}H_{64\sim72}N_2O_{17\sim18}$
Optical rotation	$[\alpha]_D^{21}$ −67.1°	$[\alpha]_D^{21}$ −71.3°
	(*c* 0.63, methanol)	(*c* 0.62, methanol)
pKa′	3.8 (70% Methyl cellosolve)	3.9 (70% Methyl cellosolve)
Titration equivalent	1,260	930
UV max: nm ($E_{1cm}^{1\%}$)		
90% MeOH	233 (154)	240 (132)
	265 (96)	265 (102)
	276 sh (82)	276 sh (93)
1 N HCl - MeOH (1: 9)	252~262 (70)	257 (86)
1 N NaOH - MeOH (1: 9)	233 (148)	239 (134)
	265 (100)	266 (106)
	276 sh (93)	275 (105)

Fig. 1. UV spectra of antlermicins B and C.

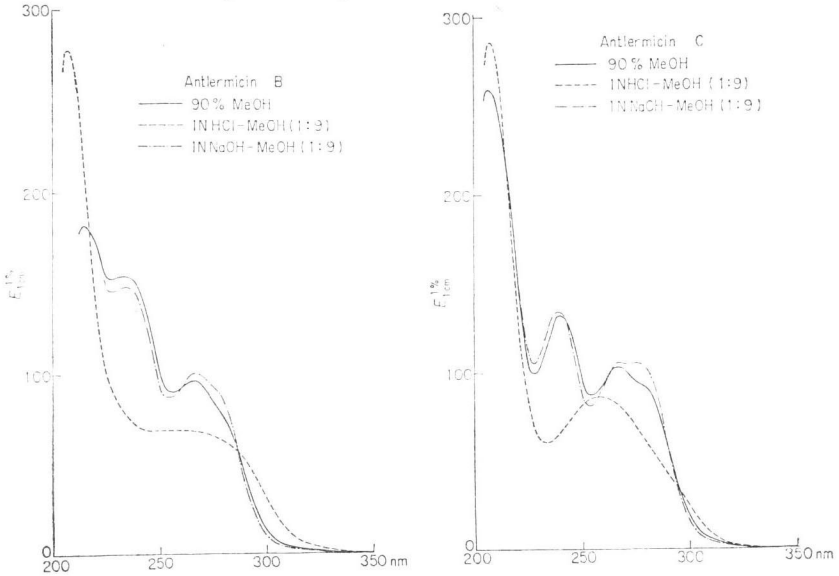

Fig. 2. IR spectra of antlermicins B and C in KBr.

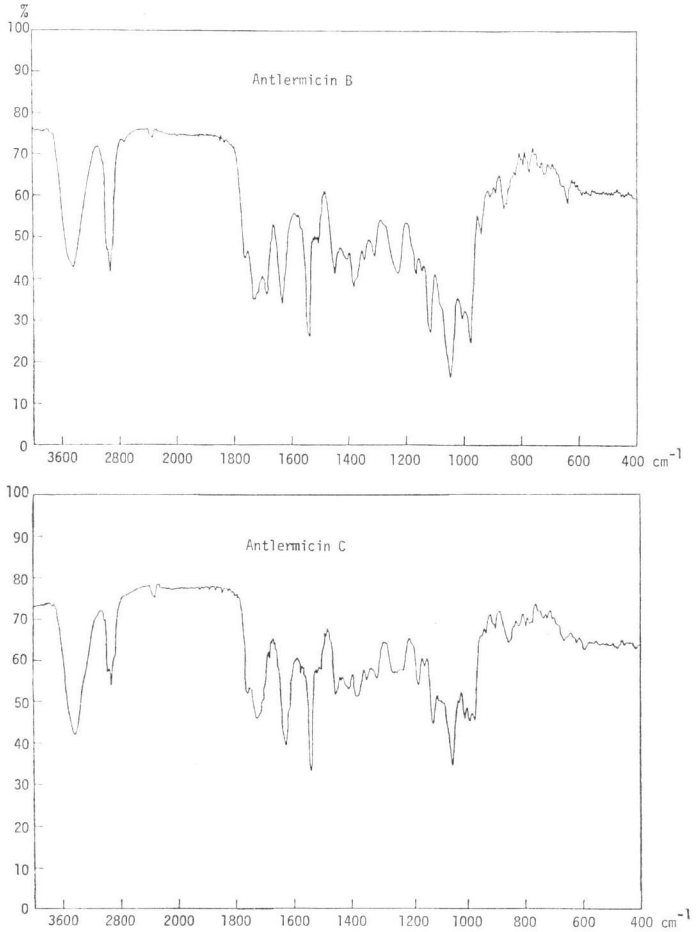

Fig. 3. ^{13}C NMR spectra of antlermicins B and C (JEOL FX 100, in acetone-d_6).

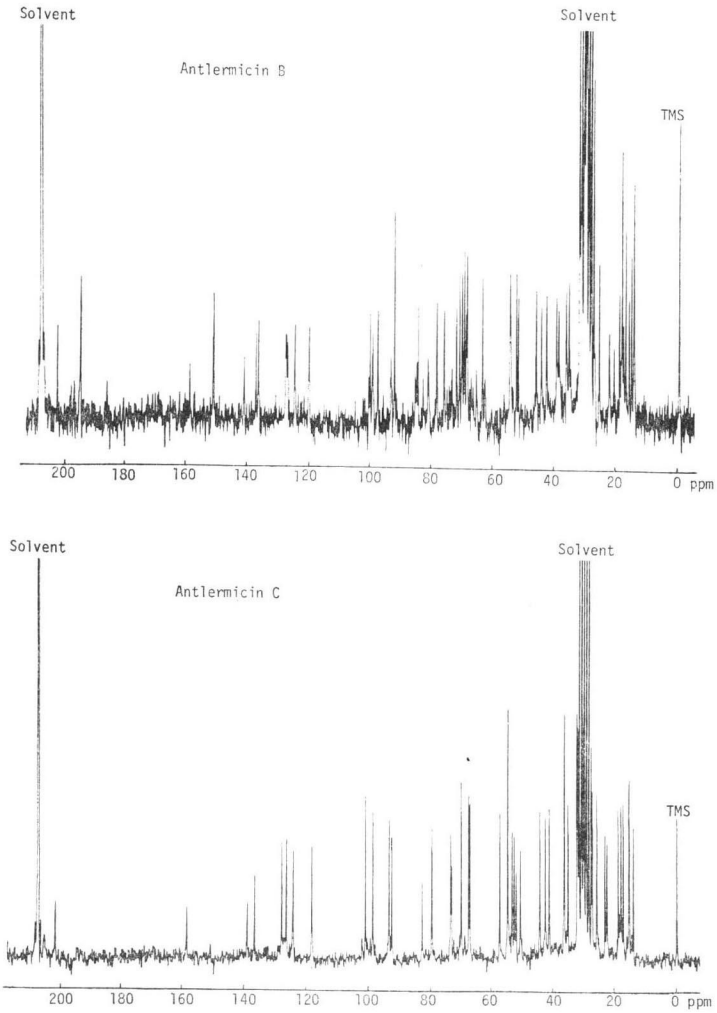

Table 2. Antimicrobial spectra of antlermicins B and C.

Microorganism	MIC (mcg/ml)	
	B	C
Bacillus subtilis PCI 219	0.05	0.78
Bacillus subtilis M45 (rec$^-$)	0.2	0.39
Bacillus cereus var. *mycoides* ATCC 11778	0.78	1.56
Bacillus agri	6.25	6.25
Micrococcus luteus	3.12	25
Staphylococcus aureus FDA 209P	>50	>50
Escherichia coli	>50	>50
Salmonella typhimurium TV 119	>50	>50
Mycobacterium phlei	>50	>50

Conventional agar-dilution method was employed using bouillon agar.

cytotoxic to YOSHIDA sarcoma cells (B, 50% inhibition at 1.56 mcg/ml; C, 30% inhibition at 12.5 mcg/ml). Mice tolerated 50 and 100 mg/kg of antlermicins B and C respectively, when injected intraperitoneally. Preliminary data indicated that antlermicins induce cell differentiation of mouse erythroid and myeloid leukemic cells and prolongation of survival period in some experimental mice tumors.

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Reference

1) KOBINATA, K.; M. URAMOTO, T. MIZUNO & K. ISONO: A new antibiotic, antlermicin A. J. Antibiotics 33: 244~246, 1980