Communications to the editor

SENACARCIN A, A NEW ANTITUMOR ANTIBIOTIC PRODUCED BY *STREPTOMYCES ENDUS* SUBSP. *AUREUS*

Sir:

We have isolated a new antitumor antibiotic, senacarcin A, from fermentation liquors of a species of *Streptomyces*. In this communication we report the production, isolation and physical properties of senacarcin A.

The producing organism was isolated from a soil collected in Sakai, Osaka, Japan. The strain has been characterized taxonomically and has been designated *Streptomyces endus* subsp. *aureus* (NRRL 12174).

The seed medium contained 20 g dextrin, 10 g glucose, 10 g peptone (Kyokuto Co.), 1 g yeast extract (Daigo Eiyo Co.), 0.5 g $MgSO_4 \cdot 7H_2O$ and 1 g calcium carbonate per liter of deionized water (pH 7.2 prior to sterilization). It was inoculated with the stock culture and incubated on a rotary shaker for 48 hours at 28°C. The seed culture was inoculated into the production medium at a rate of 5% (v/v).

The production medium contained 40 g fructose, 1 g yeast extract (Kyokuto Co.), 2.2 g L-glutamic acid monosodium salt, 0.8 g L-histidine·HCl, 1 g K_2HPO_4, 5 g $MgSO_4 \cdot 7H_2O$, 10 mg $ZnSO_4 \cdot 7H_2O$, 10 mg $CaCl_2 \cdot 2H_2O$, 10 mg $FeSO_4 \cdot 7H_2O$, 0.06 mg $CoCl_2 \cdot 6H_2O$ and 5 g $CaCO_3$ (pH 7.2 prior to sterilization). The antibiotic was detected by paper disc assays against *B. subtilis* on agar plate. The peak titers were usually reached after 3 days incubation at 28°C.

The broth was adjusted to pH 6 with sulfuric acid and filtered. The filtrate was applied to a column of Diaion HP 20 (Mitsubishi Kasei Co.),

Fig. 1. UV absorption spectrum of senacarcin A in 95% ethanol.

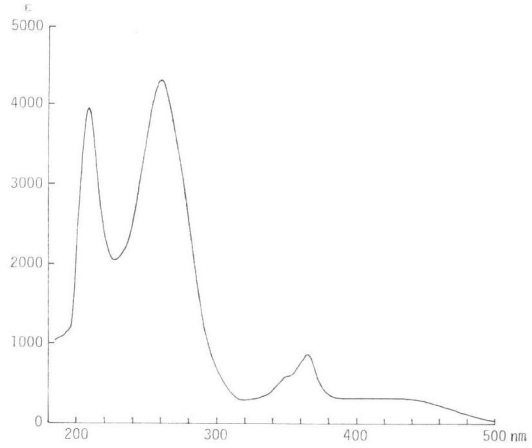


Fig. 2. IR spectra of senacarcin A (**1**) and senacarcinol (**2**) (KBr pellets).

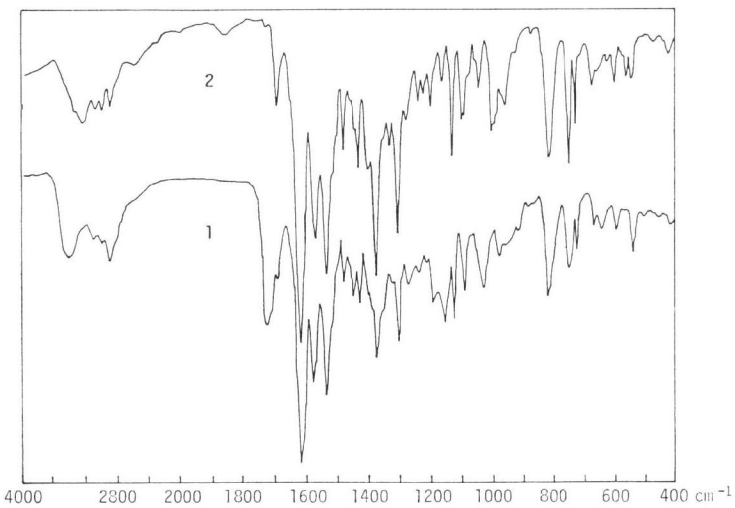
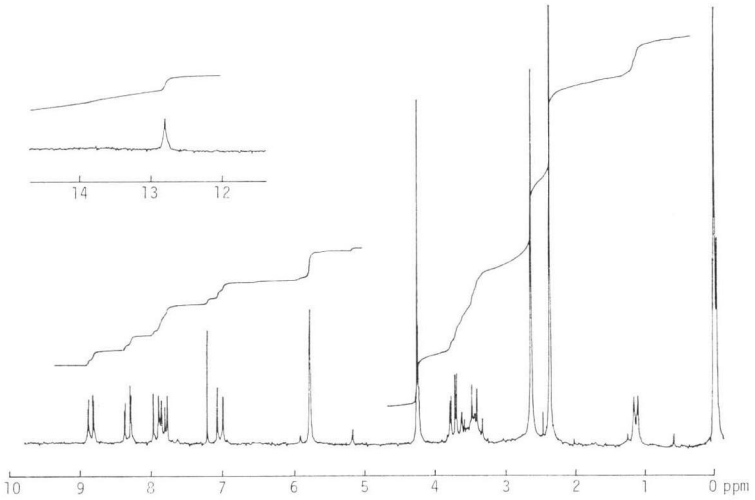


Fig. 3. ^{1}H NMR spectrum of senacarcin A in $CDCl_3$ (100 MHz).



the column was washed with deionized water and deionized water-methanol (6: 4), then eluted with methanol. The active fractions were combined, and evaporated to dryness. The yellow residue was dissolved in chloroform and silica gel was added to the solution. The solvent was removed on a rotary evaporator so that the residue was adsorbed onto the silica gel. This silica gel was applied to a column of silica gel which was slurry-packed with ethyl acetate. The antibiotic was eluted by a linear gradient of methanol in ethyl acetate (0: 10~1: 10). The active fractions were combined and concentrated to give a yellow solid. The solid was dissolved in chloroform from which yellow needles were obtained by adding ethyl acetate and cooling. In one case, 30 liters of the culture broth yielded 42 mg of senacarcin A.

Senacarcin A obtained as needles showed the following properties: mp 190°C; soluble in chloroform, methanol, acetone and H_2O but insoluble in ethyl ether and *n*-hexane; UV $\lambda_{max}^{95\%EtOH}$ 210, 263 and 366 nm (Fig. 1); IR ν_{max}^{KBr} 3400, 3290, 1730, 1690 and 1620 cm^{-1} (Fig. 2); FD-MS *m/z* 495.1791 (M+1) (Calcd. for $C_{25}H_{27}O_7N_4$: 495.1879), 379 (M+1$-C_5H_{10}O_2N$). ^{1}H NMR spectrum ($CDCl_3$, Fig. 3) showed 5 aromatic protons and 18 aliphatic protons along with 2 protons (12.8 and about 14 ppm).

Senacarcin A decomposed in alkaline solution and gave an orange colored compound, senacarcinol. Senacarcinol showed the following properties: mp 282~290°C (dec.); EI-MS *m/z* 379.1181 (M^+) (Calcd. for $C_{20}H_{17}O_5N_3$: 379.1168) 267.0750 ($M-C_5H_6O_2N$), 240.0903 ($M-C_6H_5O_3N$); UV $\lambda_{max}^{95\%EtOH}$ 215, 263, 350sh and 366 nm;

Table 1. Antimicrobial activity of senacarcin A.

Test organism	MIC (μg/ml)
Staphylococcus aureus ATCC 6538P	0.8
Bacillus subtilis No. 10707	0.4
Klebsiella pneumoniae ATCC 10031	50
Escherichia coli ATCC 26	50
Shigella sonnei ATCC 9290	25

Medium: nutrient agar (Eiken Chemical Co., Ltd.,).

Table 2. Antitumor activity of senacarcin A against murine sarcoma 180 (s.c.-i.p.).

Compound	Dose (mg/kg/day)	Treatment schedule	T/C*
Senacarcin A	25	once, day 1	Toxic
	12.5	〃	0.63
	6.25	〃	0.91
	3.13	〃	0.59
	9.38	every day; days 1~6	0.33
	6.25	〃	0.70
	3.13	〃	0.69
	1.56	〃	0.51
Mitomycin C	4	once, day 1	0.50

* T/C represents the ratio of median tumor volume of the treated group divided by that of the control group.

IR ν_{max}^{KBr} 3250~2900, 1690 and 1620 cm^{-1} (Fig. 2). ^1H NMR of senacarcinol ($CDCl_3$) showed signals at 13.76 (1H, s), 12.76 (1H, b.s), 5.30 (2H, m), 4.30 (3H, s) and 2.67 (4H, m) along with 5 aromatic protons. These data suggested that senacarcinol has a structure which has lost a $C_5H_{10}O_2N$ moiety from senacarcin A.

Senacarcin A has a similar UV spectrum to that of griseolutein A, but its molecular formula is different from that of griseolutein A[1~3] or other known phenazine antibiotics[4]. Studies on ^{13}C NMR and structure determination of senacarcin A will be reported in due course.

Senacarcin A is active mainly against Gram-positive bacteria (Table 1). Senacarcin A exhibited an inhibitory effect against sarcoma 180 in *dd*Y mice. When 9.38 mg/kg/day of senacarcin A was injected intraperitoneally once daily for 6 days, T/C was 0.33 (Table 2).

From the above data we infer that senacarcin A is a new antitumor antibiotic which has a phenazine skeleton with unique side chains.

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