DC 107, A NOVEL ANTITUMOR ANTIBIOTIC PRODUCED BY A *STREPTOMYCES* SP.

Sir:

We have screened microorganisms, isolated from soil and plants for their ability to produce antitumor antibiotics, and now have isolated a novel antitumor antibiotic DC 107 which has the molecular formula $C_{22}H_{26}N_2O_6S_3$ from a culture broth of a streptomycete. In this communication, we report the production, isolations and characterization of DC 107.

The producing organism was isolated from a soil collected in Natori-shi, Miyagi, Japan and was taxonomically classified as *Streptomyces* sp. The seed medium contained glucose 10 g, soluble starch 10 g, Bacto-tryptone 5 g, yeast extract 5 g, beef extract 3 g, $CaCO_3$ 2 g per liter (pH 7.2 prior to sterilization). It was inoculated with a stock culture and incubated for 48 hours at 28°C. The vegetative seed culture (0.9 liter) was used to inoculate into a 30-liter jar fermentor containing 18 liters of medium consisting of soluble starch 50 g, soybean meal 20 g, $CaCO_3$ 5 g, KH_2PO_4 5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g and antiform agents LG 109 (Asahi Denka Kogyo) and KM-70 (Shinetsu Kagaku) per liter (pH 7.0 prior to sterilization). The jar fermentor was stirred at 300 rpm with aeration at 18 liters/minute at 28°C. The antibacterial activity was measured by the paper-disc method on nutrient agar using *Bacillus subtilis* as the test organism and usually reached a maximum after 2 days incubation at 28°C.

The culture liquor was filtered and the filtrate, which was adjusted to pH 4.0 with AcOH, was applied to a column of Diaion HP-20 (Mitsubishi Chemical Industries Limited). The column was washed with deionized water - MeOH - AcOH (5 : 5 : 0.02) and eluted with MeOH - AcOH (10 : 0.02). The active fractions were combined, diluted with equal volume of deionized water and then applied to a column of Diaion HP20ss. The column was washed with deionized water - MeOH - AcOH (5 : 5 : 0.02) and antibiotic was eluted with deionized water - MeOH - AcOH (2 : 8 : 0.02). The active eluate was concentrated, extracted with EtOAc at pH 4.0 and concentrated to dryness. Further purification was effected by two stages of silica gel chromatography using hexane - EtOAc - AcOH

Fig. 1. IR spectrum of DC 107 (KBr).

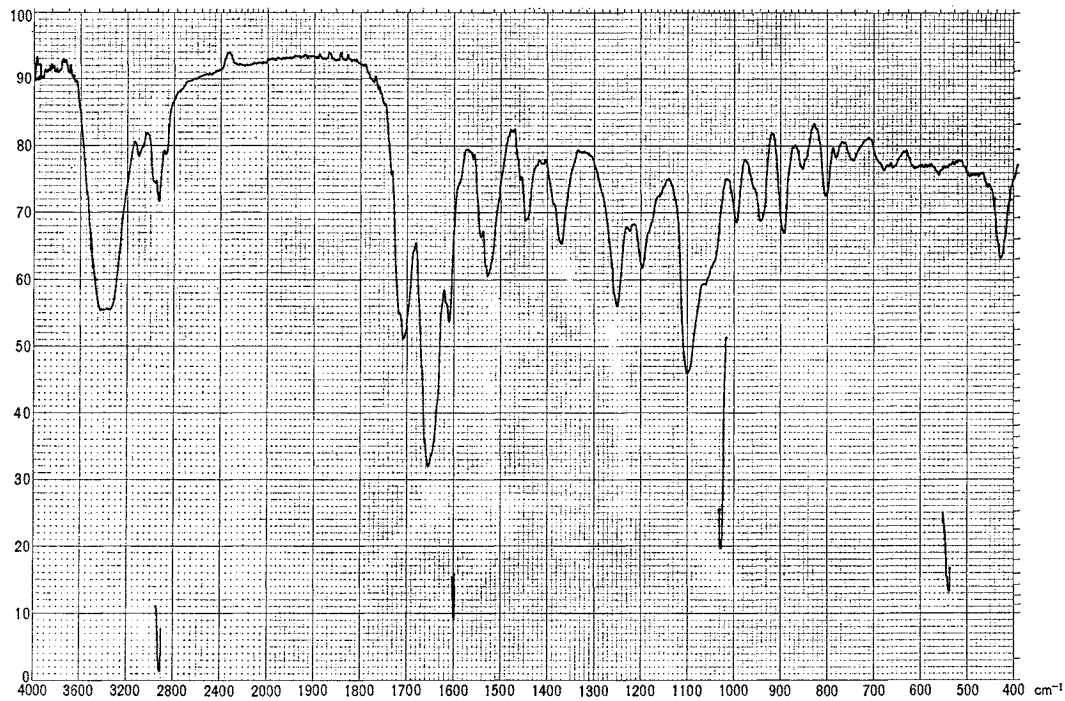
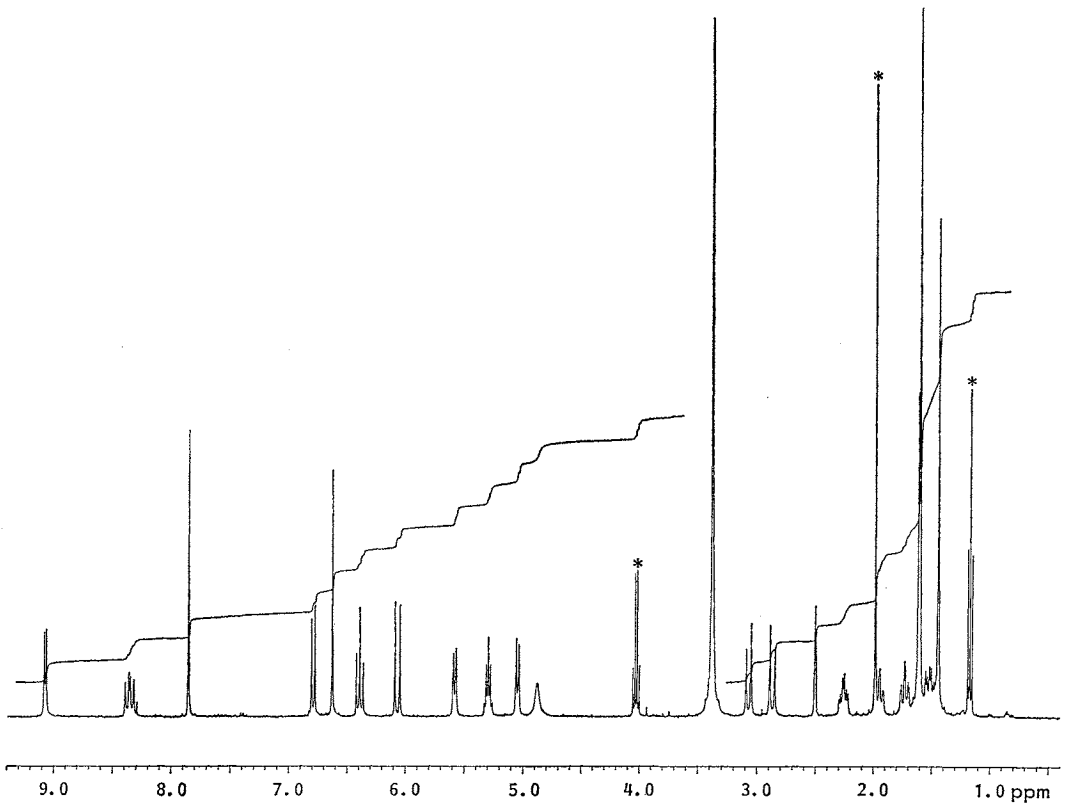


Fig. 2. ^{1}H NMR spectrum of DC 107 in DMSO-d_6 (400 MHz).
* Denotes EtOAc.



(5 : 5 : 0.1) and $CHCl_3$ - MeOH (50 : 1) as eluents to give a crude precipitate. The crude solid product was recrystallized from $CHCl_3$ to yield 50 mg of white-needled crystals of DC 107.

DC 107 showed the following physico-chemical properties: MP 155°C (dec); readily soluble in MeOH, EtOAc, $CHCl_3$, DMSO but insoluble in H_2O and *n*-hexane; UV λ_{max}^{MeOH} nm (ε) 211 (14,000), 322 (12,000); $[\alpha]_D^{25}$ −140° (*c* 0.1, MeOH); elemental analysis, calcd for $C_{22}H_{26}N_2O_6S_3 \cdot \frac{3}{4}$EtOAc: C 52.06, H 5.59, N 4.86, S 16.68; found: C 51.93, H 5.39, N 4.75, S 16.96; high-resolution fast atom bombardment mass spectroscopy (HRFAB-MS) *m/z* 511.1004 ($M^+ + 1$) (calcd for $C_{22}H_{27}N_2O_6S_3$: 511.1031). The IR spectrum of DC 107 is shown in Fig. 1. The ^{1}H NMR is given in Fig. 2. The ^{13}C NMR spectrum (Table 1) showed 22 carbon resonances. It gave a positive reaction to ninhydrin, *p*-anisidine, iodine-azide reagents, but was negative to Rydon-Smith.

It is clear from the molecular formula of DC 107, $C_{22}H_{26}N_2O_6S_3$, that the compound is a novel compound. Comparing with the antitumor antibiotic having sulfur in the molecule, *e.g.*, esperamicins[1,2], calichemicins[3], DC 107 is evidently different in UV, optical rotation, IR and NMR spectra, indicating that DC 107 is a new class of antibiotics. However, structure determination of DC 107 seems to be difficult by the spectroscopic studies, because DC 107 is a unique structure different from that of known antibiotics and is unstable to chemical degradation reactions. Effort on structure determination by X-ray analysis are in progress and will be published elswhere.

DC 107 exhibits a broad antimicrobial activity against Gram-positive and Gram-negative bacteria but not against fungi (Table 2). DC 107 was effective against murine leukemia P388 (ip), showing significant increase in life span (ILS 57%) at a dose of 0.38 mg/kg (ip) (Table 3). DC 107 also showed antitumor activity against mouse sarcoma 180 (sc), exhibiting the ratio of

Table 1. ^{13}C NMR data of DC 107 in DMSO-d_6 (100 MHz).

Chemical shift (ppm)	m
205.6	s
199.8	s
171.0	s
168.8	s
152.4	s
141.8	d
140.7	s
129.6	d
129.5	d
128.4	d
124.2	d
122.1	d
86.5	s
71.6	d
68.9	s
46.5	d
35.5	t
31.7	t
29.6	t
23.5	q
20.7	q
19.5	q

m: Multiplicity.

Table 2. Antimicrobial activity of DC 107.

Organism	MIC (μg/ml)
Staphylococcus aureus ATCC 6538P	1.6
Enterococcus faecium ATCC 10541	1.6
Bacillus subtilis No. 10707	0.08
Klebisiella pneumoniae ATCC 10031	1.6
Escherichia coli ATCC 26	2.6
Pseudomonas aeruginosa Bin H No. 1	5.2
Salmonella typhi ATCC 9992	20.8
Proteus vulgaris ATCC 6897	83.3
Shigella sonnei ATCC 9290	10.4
Candida albicans ATCC 10231	>100

Table 3. Antitumor activity of DC 107 against P388 lymphocytic leukemia in mice[a].

Dose[b] (mg/kg)	ILS (%)
1.5	49
0.75	45
0.38	57
0.19	34
0.095	23

[a] Tumor inoculated ip on day 0.
[b] Single dose given ip on day 1.

median tumor volume (T/C 41%) at a dose of 1 mg/kg (iv). The LD_{50} value of DC 107 was 2.8 mg/kg (iv) in mouse. Further detailed studies on antitumor spectra and toxicity of DC 107 are in progress.

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