Communications to the Editor

DC 102, A NEW GLYCOSIDIC PYRROLO(1,4)BENZODIAZEPINE ANTIBIOTIC PRODUCED BY *STREPTOMYCES* SP.

Sir:

We have screened microorganisms, isolated from soil and plants for their ability to produce antitumor antibiotics, and now have isolated a new glycosidic pyrrolo(1,4)benzodiazepine antibiotic named DC 102 from a cultured broth of a Streptomycete. In this communication, we report the production, isolation and characterization of DC 102.

The producing organism was isolated from a soil collected in Onuma Park, Hokkaido, Japan. 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 of deionized water (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 fermentator containing 18 liters of medium consisting of soluble starch 50 g, dry yeast 20 g, $CaCO_3$ 5 g, KH_2PO_4 5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g and antifoam agents LG 109 (Asahi Denka Kogyo) and KM-70 (Shinetsu Kagaku) per liter of deionized water (pH 7.0 prior to sterilization). The jar fermenter was stirred at 300 rpm with aeration at 18 liters/minute. 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-day incubation at 28°C.

The culture liquor was filtered and the filtrate (30 liters) was applied to a column of Diaion HP-20 (Mitsubishi Chemical Industries Limited) which was washed with deionized water. The active fractions eluted with MeOH were pooled, concd *in vacuo* to a volume of 5 liters and then applied to a column of Diaion SK-104 (NH_4^+ form). The column was washed with deionized water, and the antibiotic was eluted with 2 M ammonium acetate - MeOH (1 : 1). The active eluate was passed through a column of Diaion HP-20, the column was washed with deionized

Fig. 1. IR spectrum of DC 102 (KBr).

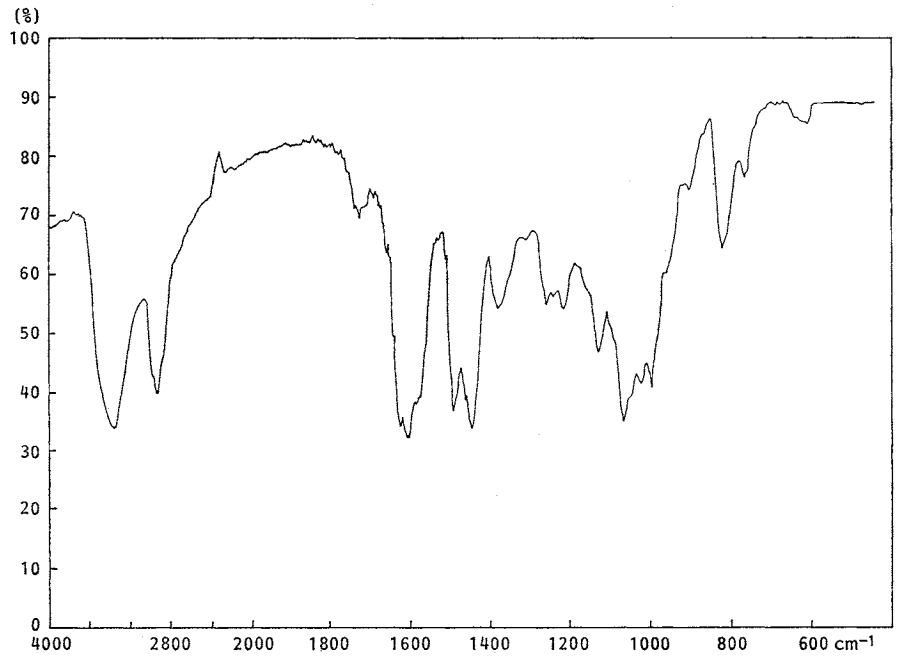


Fig. 2. ^{13}C NMR spectrum of DC 102 in CD_3OD (100 MHz).

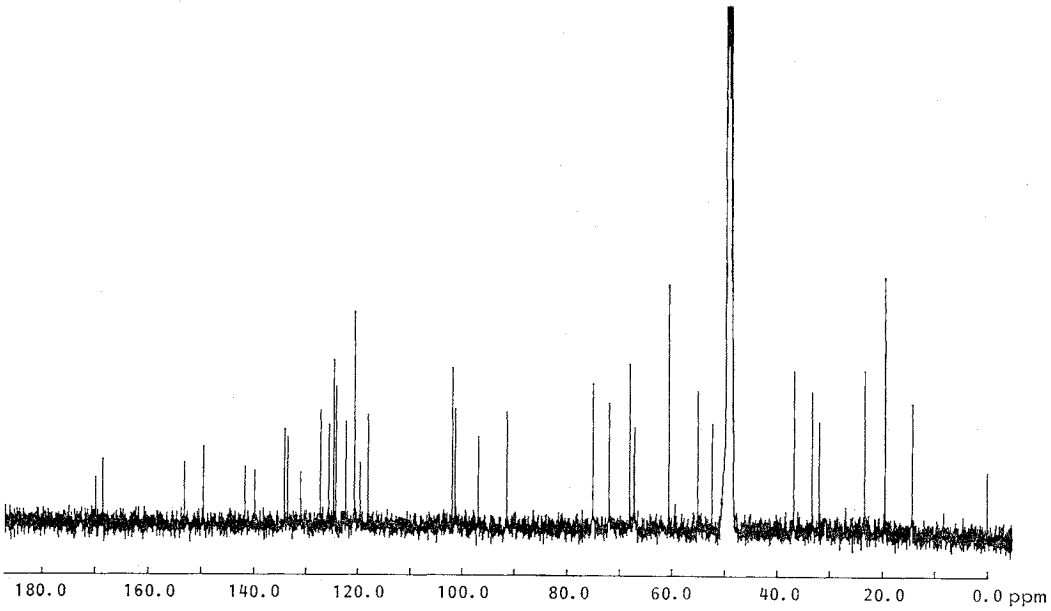
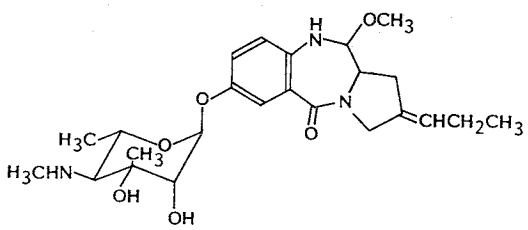


Fig. 3. Structure of DC 102.



water and eluted with MeOH. The active fractions were combined and concentrated to dryness. The residue was chromatographed on a silica gel column using $CHCl_3$ - MeOH (7:3) as eluents. The active fraction was concentrated to dryness, redissolved in MeOH and then passed through a column of DEAE-Sepharose CL-6B (Cl^- form). The effluent containing DC 102 was concentrated to dryness and further chromatographed on silica gel column using EtOAc - MeOH (20:1) as eluents to yield 5 mg of DC 102.

DC 102 is a basic compound obtained as white powder. It showed the following properties: MP 120°C (dec); readily soluble in MeOH, EtOH, slightly soluble in H_2O and Me_2CO but insoluble in $CHCl_3$, *n*-hexane; UV λ_{max}^{MeOH} nm (ε) 210 (14,000), 244 (sh, 11,000), 310 (6,500); electron impact (EI)-mass *m/z* 429.2261 (M^+ — CH_3OH) (calcd for $C_{23}H_{31}N_3O_5$: 429.2242). The IR spectrum of DC 102 is shown in Fig. 1. The ^{13}C NMR is given in Fig. 2.

DC 102 had a UV spectrum characteristic of

Table 1. Antimicrobial activity of DC 102.

Organism	MIC (μg/ml)
Candida albicans ATCC 10231	>100
Streptococcus faecium ATCC 10541	83
Pseudomonas aeruginosa Bin H No. 1	>100
Staphylococcus aureus ATCC 6538P	83
Escherichia coli ATCC 26	>100
Bacillus subtilis No. 10707	42
Proteus vulgaris ATCC 6897	>100
Shigella sonnei ATCC 9290	>100
Salmonella typhi ATCC 9992	>100
Klebsiella pneumoniae ATCC 10031	>100

Table 2. Antitumor activity of DC 102 against P388 lymphocytic leukemia in mice[a].

Compound	Dose[b]	T/C (%)
DC 102	1	140
	0.5	154
	0.25	125
	0.125	120
Mitomycin C	6	166

[a] Tumor inoculated intraperitoneally on day 0.
[b] mg/kg/injection: Single dose given intraperitoneally on day 1.

pyrrolo(1,4)benzodiazepine antibiotics[1,2]. It gave a positive reaction to *p*-anisidine, indicating the presence of a sugar moiety. However, its molecular formula is different from that of sibiromycin[3], the only aminoglycosidic pyrrolo-(1,4)benzodiazepine antibiotic so far reported. The structure of DC 102 (Fig. 3) was assigned by NMR spectroscopic studies and will be reported in a separate paper (M. YOSHIDA & H. SANO; in preparation).

DC 102 exhibited weak antimicrobial activity against Gram-positive bacteria (Table 1). DC 102 was effective against murine leukemia P388, showing significant increase of life span (ILS 54%) at a dose of 0.5 mg/kg (Table 2). The LD_{50} value of DC 102 was 1.5 mg/kg (ip) in mouse. The detailed studies of the antitumor activity of DC 102 are in progress and will be published in due course.

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