# DC89-A1, A NEW ANTITUMOR ANTIBIOTIC FROM *STREPTOMYCES*

Sir:

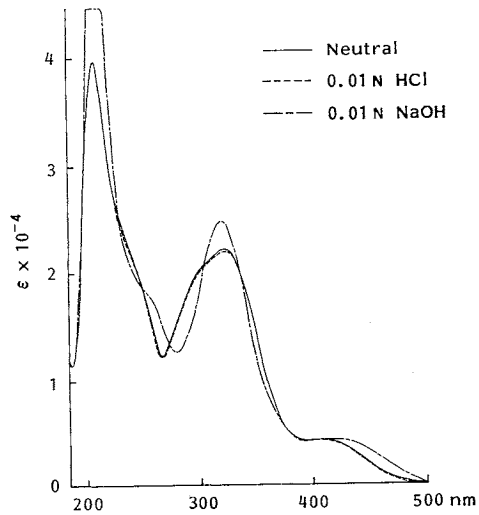
We have isolated a new antitumor antibiotic DC89-A1 which has the molecular formula $C_{26}H_{26}N_3O_8Cl$ from a culture broth of a streptomycete. In this communication, we report the production, isolation, physico-chemical and biological properties of DC89-A1.

The producing organism was isolated from a soil collected at Mt. Rokko in Hyogo, Japan, and was taxonomically classified as *Streptomyces* sp. (FERM BP 988) based on both whole cell wall analysis and morphology[1]. A seed broth was prepared by inoculating spores of the producing strain into a medium consisting of glucose 1%, soluble starch 1%, beef extract 0.3%, yeast extract 0.5%, Bacto-tryptone 0.5% and $CaCO_3$ 0.2% (pH 7.0). After incubation at 28°C for 48 hours, the vegetative seed culture (15 liters) was inoculated into a 200-liter tank containing 150 liters of the production medium consisting of dextrin 5%, dry yeast 1%, $NaNH_4HPO_4$ 1%, $KH_2PO_4$ 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05%, KCl 1%, $CaCO_3$ 0.5% (pH 7.0). The fermentor was stirred at 200 rpm with aeration at 150 liters/minute at 28°C. Total antibacterial activity reached a maximum at 57 hours, measured with a paper-disc assay on nutrient agar using *Bacillus subtilis* as test organism.

Table 1. Physico-chemical properties of DC89-A1.

| | |
|---|---|
| Appearance | Yellow powder |
| $[\alpha]_D^{20}$ | −126° (*c* 0.2, MeOH) |
| EI-MS (*m/z*) | 543 |
| HR-EI-MS | Found: 543.1406 |
| | Calcd for $C_{26}H_{26}N_3O_8Cl$: 543.1422 |
| UV $\lambda_{max}^{MeOH}$ nm (ε) | 323 (23,000), 415 (4,000) |
| IR $\nu_{max}^{CHCl_3}$ cm$^{-1}$ | 3450, 3300, 1740, 1695, 1620 |
| Solubility | Soluble: MeOH, $CHCl_3$, EtOAc, $Me_2CO$, DMSO |
| | Insoluble: Hexane, water |

Fig. 1. UV spectra of DC89-A1 in MeOH.




Scheme 1. Procedure for the isolation of DC89-A1.

Culture filtrate and $Me_2CO$ extract of mycelium
↓
Diaion HP-20
↓ elute with MeOH
↓ concentrate
Extraction with EtOAc
↓ concentrate
Silica gel (Wakogel C-200)
↓ elute with *n*-hexane - EtOAc - AcOH; gradient of (600 : 300 : 5) to (400 : 950 : 6)
↓ concentrate
Silica gel (LiChroprep Si 60)
↓ elute with *n*-hexane - EtOAc - AcOH; gradient of (160 : 40 : 1) to (80 : 120 : 1)
↓ concentrate
Aminopropyl silica gel (Wakogel LC $NH_2$-10H)
↓ elute with gradient of $CH_3CN$ to $CH_3CN$ - $H_2O$ (9 : 1)
↓ concentrate
DC89-A1 (yellow powder)

Fig. 2. IR spectrum of DC89-A1 ($CHCl_3$).

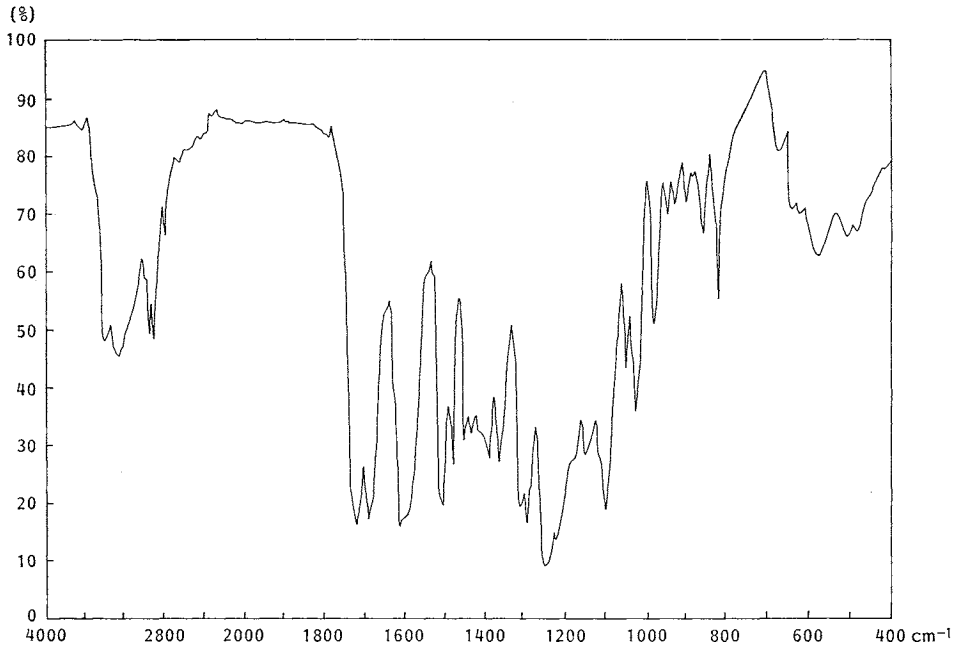



Fig. 3. $^1H$ NMR spectrum of DC89-A1 in DMSO-$d_6$ with internal TMS at 400 MHz.

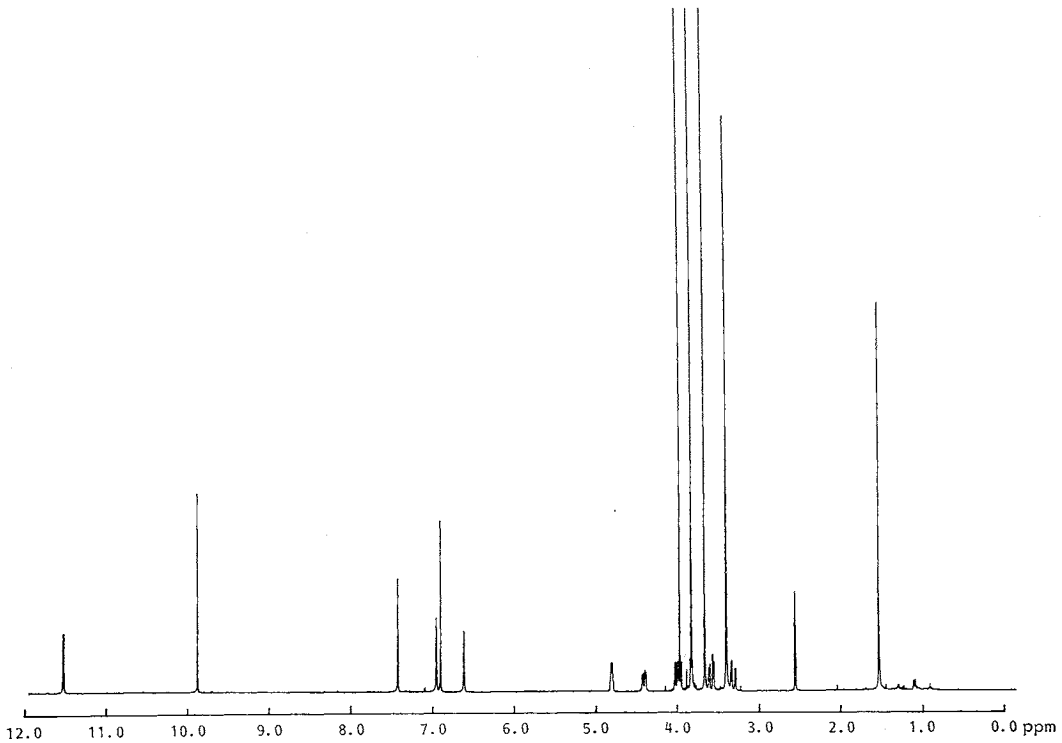
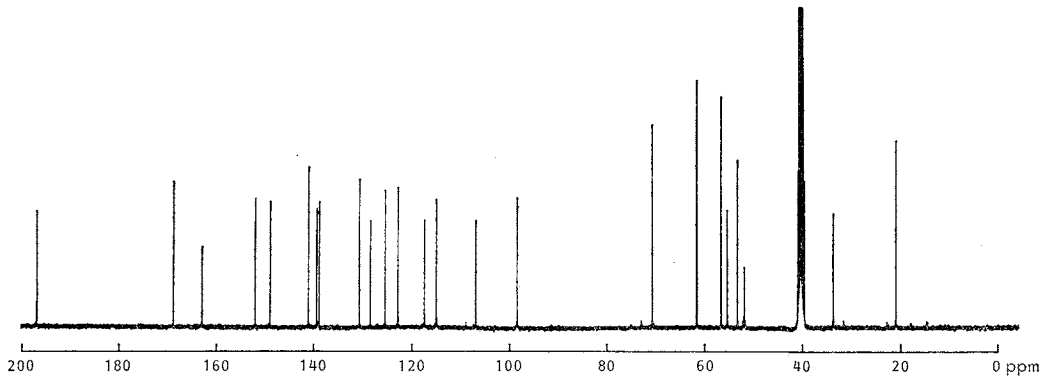


Fig. 4. $^{13}C$ NMR spectrum of DC89-A1 in DMSO-$d_6$ with internal TMS at 100 MHz.




DC89-A1 was isolated from the culture broth by the steps presented in Scheme 1. The antibacterial activity was detected in both mycelium and extra cellular medium. The combined culture filtrate and acetone extract of mycelium was adjusted to pH 5.5 with sulfuric acid and was applied to a column of Diaion HP-20 (Mitsubishi Chemical Industries Limited). The column was washed with water and then the antibiotic was eluted with MeOH. The eluate was concentrated *in vacuo* and extracted with EtOAc. The extracts were concentrated and the residue was subjected to silica gel chromatography twice using a mixture of *n*-hexane - EtOAc - AcOH as an eluting solvent. The active fractions were combined and evaporated to dryness. The residue was chromatographed on an aminopropyl silica gel (Wakogel LC $NH_2$-10H, Wako Junyaku) using water - acetonitrile and after concentration, 10 mg, of pure DC89-A1 was obtained as yellow powder.

The physico-chemical properties of DC89-A1 are summarized in Table 1. DC89-A1 displayed good solubility in DMSO, MeOH, $CHCl_3$, EtOAc and slight solubility in hydrocarbons and water. A MeOH solution of DC89-A1 exhibited strong absorption at 323 nm ($\varepsilon$ 23,000) and a maximum at 415 nm ($\varepsilon$ 4,000) as shown in Fig. 1. The IR spectrum (Fig. 2) was consistent with the presence of NH and OH (3450 and 3300), ester (1740), carbonyl (1695) and amide group (1620). The molecular weight of DC89-A1 was determined as 543 from electron impact (EI)-MS data. The high-resolution electron impact mass spectra (HR-EI-MS) of the compound showed molecular ions at *m/z* 543.1422 and 545.1355 (calcd for $C_{26}H_{26}N_3O_8{}^{35}Cl$: 543.1406 and $C_{26}H_{26}N_3O_8{}^{37}Cl$: 545.1376), indicating the molecular formula of DC89-A1 to be $C_{26}H_{26}N_3O_8Cl$. $^1H$ NMR spectrum (Fig. 3) displayed four methoxyl proton signals, phenolic

Table 2. Antimicrobial activity of DC89-A1.

| Organism | MIC ($\mu$g/ml) |
|---|---|
| *Staphylococcus aureus* ATCC 6538P | 0.03 |
| *Enterococcus faecium* ATCC 10541 | 0.08 |
| *Bacillus subtilis* No. 10107 | 0.01 |
| *Escherichia coli* ATCC 26 | 42 |
| *Klebsiella pneumoniae* ATCC 10031 | 0.16 |
| *Proteus vulgaris* ATCC 6897 | 20 |
| *Shigella sonnei* ATCC 9290 | 83 |
| *Salmonella typhi* ATCC 9992 | 83 |
| *Pseudomonas aeruginosa* Bin H No. 1 | 83 |
| *Candida albicans* ATCC 10231 | 83 |

Table 3. Antitumor activity of DC89-A1.

(A) P388 lymphocytic leukemia in CDF1 mice:

| Compound | Dose (mg/kg) | T/C (%) |
|---|---|---|
| DC89-A1 | 0.50 | 144 |
| | 0.25 | 138 |
| | 0.12 | 133 |
| | 0.06 | 127 |
| Mitomycin C | 2.0 | 152 |

Single dose given ip on day 1 after tumor inoculation.

(B) Sarcoma 180 in *dd*Y mice:

| Compound | Dose (mg/kg) | T/C (%) |
|---|---|---|
| DC89-A1 | 12 | 0.03 (2/5*) |
| | 8 | 0.17 |
| | 6 | 0.28 |
| | 4 | 0.49 |
| Mitomycin C | 4 | 0.34 |

Single dose given iv on day 1 after tumor inoculation.

* Mortality.

hydroxyl proton, amino proton and three aromatic proton signals. The $^{13}C$ NMR spectrum (Fig. 4) showed twenty six carbon signals. Studies on structure determination of DC89-A1 are in progress and will be published in due course.

DC89-A1 showed antimicrobial activity, mainly against Gram-positive bacteria (Table 2). The single dose $LD_{50}$ in *dd*Y mice was 14 mg/kg with iv administration and 1.2 mg/kg by ip route. The activity of DC89-A1 against mouse tumor systems[2] is summarized in Table 3. DC89-A1 was effective against murine lymphocytic leukemia P388 transplanted in CDF1 mice, showing significant increase of life span at a single ip dose of 0.50~0.06 mg/kg. The compound also showed a strong antitumor activity against murine sarcoma 180 in *dd*Y mice; T/C= 0.17 and 0.28 at single iv doses of 8 mg/kg and 6 mg/kg, respectively. The results of our work show that DC89-A1 is a new antibiotic with high antitumor potency. Further detailed studies on antitumor spectra and toxicity of DC89-A1 are in progress.

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