DUOCARMYCIN A, A NEW ANTITUMOR ANTIBIOTIC FROM *STREPTOMYCES*

Sir:

We have isolated a potent new antitumor antibiotic duocarmycin A from a culture broth of a Streptomycete. In this communication, we report the production, isolation, physico-chemical and biological properties of duocarmycin A (also known as DC88-A).[1]

The producing organism was isolated from a soil collected at the foot of Mt. Fuji in Shizuoka, Japan and was taxonomically classified as *Streptomyces* sp. DO-88 (FERM BP 1002). A seed broth was prepared by inoculating spores of the producing strain into a medium consisting of glucose 1%, dextrin 2%, peptone 1%, yeast extract 1%, corn steep liquor 0.5% and $CaCO_3$ 0.2% (pH 7.2). After incubation at 28°C for 48 hours, the vegetative seed culture (300 ml) was inoculated into a 30-liter jar containing 15 liters of the production medium consisting of soluble starch 2%, dry yeast 0.5%, KH_2PO_4 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05%, trace metal dry mixture 0.0088%[2] and $CaCO_3$ 0.5% (pH 7.0). The fermentor was stirred at 300 rpm with aeration at 15 liters/minute at 28°C. The pH of medium was adjusted to 6.5~6.8 with aqueous ammonia during cultivation. Total antibacterial activity reached a maximum at 72 hours, measured with a paper-disc assay on nutrient agar using *Bacillus subtilis* as the test organism.

Duocarmycin A was isolated from the culture broth by the following steps. The antibacterial activity was detected in both mycelium and extra cellular medium. The combined culture filtrate and acetone extract of the mycelium (30 liters) 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 50% methanol, and then the antibiotic was eluted with ethyl acetate. The eluate was concentrated *in vacuo* and extracted with ethyl acetate. The extract was concentrated and the residue was subjected to silica gel chromatography using a mixture of toluene - acetone as an eluting solvent. The active fractions were combined and evaporated to dryness. The residue was rechromatographed on silica gel (Lichroprep Si 60, Merck) with toluene - acetone. The crude product was subjected to HPLC (Wako gel-LC-ODS 30K) with a gradient elution (50% aqueous methanol to methanol) to yield 3 mg of pure duocarmycin A.

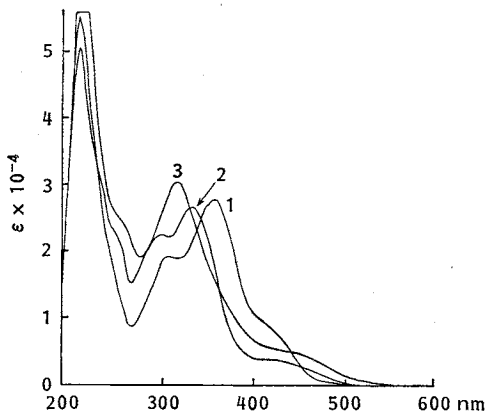
The physico-chemical properties of duocarmycin A are summarized in Table 1. Duocarmycin A was obtained as a yellow powder and displayed good solubility in dimethyl sulfoxide, methanol, chloroform and ethyl acetate and slight solubility in hydrocarbons and water. A methanol solution of duocarmycin A exhibited strong absorption at 358 nm which showed characteristic shifts under acidic and alkaline conditions as shown in Fig. 1. The IR spectrum

Table 1. Physico-chemical properties of duocarmycin A.

Appearance	Yellow powder
MP	147~148°C
$[\alpha]_D^{22}$	+282° (*c* 0.1, MeOH)
HREI-MS	
Calcd for $C_{26}H_{25}N_3O_8$:	507.1639
Found:	507.1624
UV λ_{max}^{MeOH} nm (ε)	310 (18,000), 358 (28,000), 425 (sh, 8,000)
IR $\nu_{max}^{CHCl_3}$ cm^{-1}	3600, 3450, 3300, 1740, 1684, 1630
^1H NMR (400 MHz, $CDCl_3$, internal standard; TMS) δ	9.49 (1H, br s), 7.17 (1H, s), 6.94 (1H, d), 6.78 (1H, s), 6.36 (1H, br s), 4.45, 4.41 (AB in ABX), 4.06 (3H, s), 3.93 (3H, s), 3.88 (3H, s), 3.74 (3H, s), 3.05 (1H, m), 2.24 (1H, dd), 1.67 (3H, br s), 1.29 (1H, dd)
^{13}C NMR (100 MHz, $CDCl_3$) δ	194.8, 179.7, 168.0, 165.1, 164.4, 161.2, 150.6, 141.3, 138.9, 128.2, 126.6, 123.3, 113.2, 112.0, 108.2, 97.7, 71.3, 61.5, 61.2, 56.3, 55.3, 53.4, 30.6, 22.3, 22.0, 21.1
Solubility	
Soluble:	MeOH, $CHCl_3$, EtOAc, Me_2CO, DMSO
Insoluble:	Hexane, water

indicates the presence of NH (3450 and 3300), ester (1740), ketone (1684) and amide groups (1630 cm^{-1}). The molecular weight of duocarmycin A was determined as 507 from mass spectral data. The high resolution electron impact mass spectrum (HREI-MS) of the compound showed molecular ion at *m/z* 507.1624 indicating the molecular formula of duocarmycin A to be $C_{26}H_{25}N_3O_8$ (calcd 507.1639). The ^1H NMR spectrum displayed four methoxyl proton, two amino proton, one tertiary methyl and three aromatic proton signals. The ^{13}C NMR spectrum showed twenty six carbon signals: 15 quaternary, 4 methine, 2 methylenes, 4 methoxyl and 1 methyl.

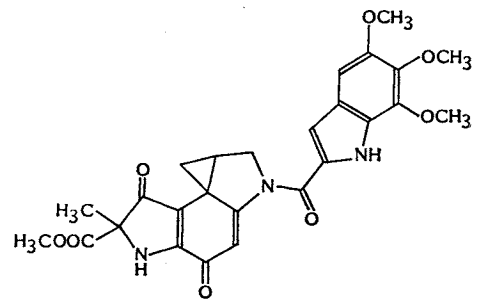
Fig. 1. UV spectra of duocarmycin A in MeOH.
1: Neutral, 2: 0.01 N HCl, 3: 0.01 N NaOH.



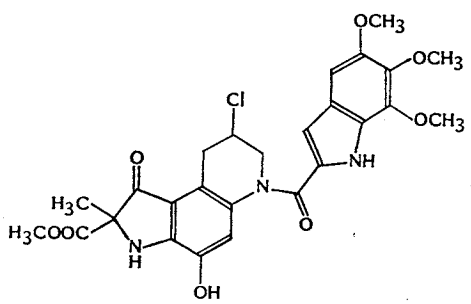
From an analysis of the ^1H and ^{13}C NMR spectra of duocarmycin A together with the mass spectral evidence, it was apparent that duocarmycin A and DC89-A1[3] are close structural analogs. A number of differences were also noted, especially the presence of Cl in DC89-A1 and not in duocarmycin A. The structures of duocarmycin A and DC89-A1 were assigned as shown in Fig. 2 by YASUZAWA *et al.*, which will be reported in a separate paper.[4] Duocarmycin A possess a unique structure with DNA alkylating capability which has been reported in the cyclopropylpyrroloindole "left hand segment" of CC-1065.[5,6]

Duocarmycin A showed strong antimicrobial activity against Gram-positive bacteria (Table 2).

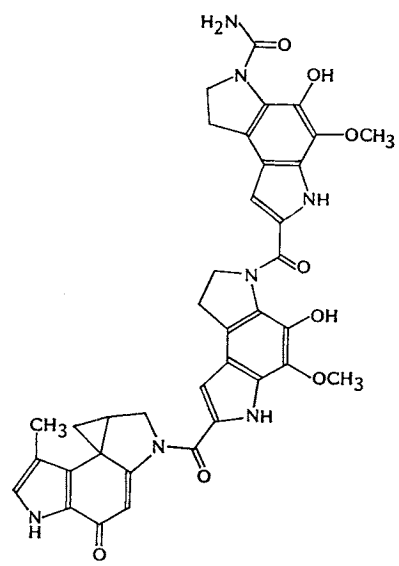
Fig. 2. Structures of duocarmycin A, DC89-A1 and CC-1065.



Duocarmycin A



DC89-A1



CC-1065

Table 2. Antimicrobial properties of duocarmycin A.

Organism	MIC (μg/ml)
Staphylococcus aureus ATCC 6538P	0.0064
Enterococcus faecium ATCC 10541	0.0016
Bacillus subtilis #10107	0.0064
Klebsiella pneumoniae ATCC 10031	0.032
Escherichia coli ATCC 26	4.2
Pseudomonas aeruginosa BinH#1	42
Salmonella typhi ATCC 9992	4.2
Proteus vulgaris ATCC 6897	1.0
Shigella sonnei ATCC 9290	4.2
Candida albicans ATCC 10231	1.0

The MICs against Gram-positive bacteria are under 0.01 μg/ml, while values for Gram-positive bacteria and yeast are mostly between $1\sim10$ μg/ml. The single dose LD_{50} in *dd*Y mice was 0.034 mg/kg with iv administration. Duocarmycin A was effective against murine lymphocytic leukemia P388 transplanted in CDF1 mice, showing a significant increase in life span (data not shown). The compound also showed strong antitumor activity against murine sarcoma 180 in *dd*Y mice: T/C=0.35 at single ip doses of 0.04 mg/kg, T/C=$0.26\sim0.42$ at single iv dose of $0.03\sim0.0075$ mg/kg. The results of our work show that duocarmycin A is a new antibiotic with high antimicrobial and antitumor potency. Further detailed studies on antitumor spectra and toxicity of duocarmycin A are in progress.

Addendum in Proof

After our report on DC89-A1,[3] K. OHBA *et al.* reported pyrindamycins A and B (J. Antibiotics 41: $1515\sim1519$, 1988). The presented data indicated that pyrindamycin B is identical to DC89-A1 which is also named duocarmycin C1.[4]

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References

1) NAKANO, H.; I. TAKAHASHI, M. ICHIMURA, I. KAWAMOTO, K. ASANO, F. TOMITA, H. SANO, T. YASUZAWA, M. MORIMOTO & K. FUJIMOTO (Kyowa Hakko): Novel compounds DC88-A and DC89-A1 and process for their preparation. PCT. Int. Appl. 06265 ('87), Oct. 22, 1987
2) HUNTER, S. H.: Inorganic nutrition. Annu. Rev. Microbiol. 26: $313\sim346$, 1972
3) ICHIMURA, M.; K. MUROI, K. ASANO, I. KAWAMOTO, F. TOMITA, M. MORIMOTO & H. NAKANO: DC89-A1, a new antitumor antibiotic from *Streptomyces*. J. Antibiotics 41: $1285\sim1288$, 1988
4) YASUZAWA, T.; T. IIDA, M. ICHIMURA, K. MUROI, K. TAKAHASHI & H. SANO: Structure determination of duocarmycins, novel antitumor antibiotics produced by *Streptomyces* sp. Chem. Pharm. Bull. 36: $3728\sim3731$, 1988
5) MARTIN, D. G.; C. G. CHIDESTER, D. J. DUCHAMP & S. A. MIZSAK: Structure of CC-1065 (NSC-298223), a new antitumor antibiotic. J. Antibiotics 33: $902\sim903$, 1980
6) ADAMS, E. G.; G. J. BADINER & B. K. BHUYAN: Effect of U-71184 and several other CC-1065 analogues on cell survival and cell cycle of chinese hamster ovary cells. Cancer Res. 48: $109\sim116$, 1988