DUOCARMYCIN SA, A NEW ANTITUMOR ANTIBIOTIC FROM *STREPTOMYCES* SP.

Sir:

We have isolated a potent new antitumor antibiotic DC113 from a culture broth of a streptomycete. DC113 is structurally related to duocarmycin A[1], and more stable than duocarmycin A. DC113 shows antibacterial and cytotoxic activities at lower concentrations than those of duocarmycin A, thereby is named duocarmycin SA. In this communication, we report the production, isolation, physico-chemical and biological properties of duocarmycin SA.

The producing organism was isolated from soil collected at the Rokkakudo temple in Kyoto, Japan and was taxonomically classified as *Streptomyces* sp. DO113 (FERM BP-222). A seed broth was prepared by inoculating spores of the producing strain into a medium consisting of yeast extract 0.5%, Bacto Tryptone 0.5%, glucose 1%, soluble starch 1%, beef extract 0.3% and $CaCO_3$ 0.2% (pH 7.2). After incubation at 28°C for 48 hours, a 5%-vegetative seed culture was inoculated into fermentation medium consisting of soluble starch 5%, dry yeast 1.4%, KH_2PO_4 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05%, $CuSO_4$ (anhydrous) 0.0001%, $CrK(SO_4)_2 \cdot 12H_2O$ 0.0001%, $NiSO_4 \cdot 6H_2O$ 0.00005% and $CaCO_3$ 0.5% (pH 7.0). Total antibacterial activity was detected by paper disc assay against *Bacillus subtilis* on agar plate. The peak titers were usually reached after 4 days incubation at 28°C.

Duocarmycin SA was isolated from the culture broth by the following steps. The antibacterial activity was detected in both mycelium and extracellular medium. Propanol (550 liters) was added into the culture broth (1,100 liters) and the mixture was filtrated. The filtrate was diluted by deionized water (150 liters) and was applied to a column of Diaion HP-20 (50 liters) (Mitsubishi Chemical Industries Limited). The column was washed with deionized water and 30% propanol and then the antibiotic was eluted with ethyl acetate. The eluate was concentrated and extracted with ethyl acetate. The extract was concentrated and the residue was subjected to silica gel (Merck Art. No. 7734) column chromatography using a mixture of hexane-ethyl acetate and then ethyl acetate-methanol as eluting solvents. The active fractions were combined and evaporated to dryness. The residue was rechromatographed on an aminopropyl silane (NH_2) silica gel (J. T. BAKER, Chemical Co.) with toluene-acetone, and the active fractions were further purified with HPLC using a packed column (YMC-ODS SH-363-5, MeOH-H_2O, 7:3) to yield 16.2 mg of pure duocarmycin SA.

The physico-chemical properties of duocarmycin SA are summarized in Table 1. Duocarmycin SA was obtained as an optically active pale yellow powder, $[\alpha]_D^{24}$ +180° in MeOH. The molecular formula of duocarmycin SA was determined as $C_{25}H_{23}N_3O_7$ (Calcd 477.1534) by HREI-MS which showed molecular ion at *m/z* 477.1522.

The UV absorption maxima and the IR spectrum of duocarmycin SA are similar to those of duocarmycin type antibiotics[1~6]. The structure of duocarmycin SA was assigned by NMR spectroscopic studies and was shown to be different from that of duocarmycin A at pyrrolinone moiety (Fig. 1). Details of structure determination will be reported elsewhere. Duocarmycin SA as well as duocarmycin A possess a unique structure with DNA alkylating capability which has been reported in the cyclopropapyrroloindol "left hand segment" of CC-1065[7,8]. Duocarmycin SA is more stable than duocarmycin A under various conditions. It is considered that the stability of duocarmycin SA might be resulted from its pyrrole structure.

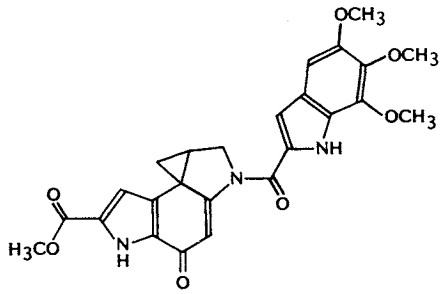
Duocarmycin SA shows the potent antimicrobial activities against Gram-positive bacteria. The MICs of duocarmycin SA against *Staphylococcus aureus* and *Bacillus subtilis* are 0.0013 μg/ml and 0.00065 μg/ml, respectively, which are lower than those of duocarmycin A. The single dose LD_{50} in *dd*Y mice was 0.143 mg/kg with iv administration. Duocarmy-

Table 1. Physico-chemical properties of duocarmycin SA.

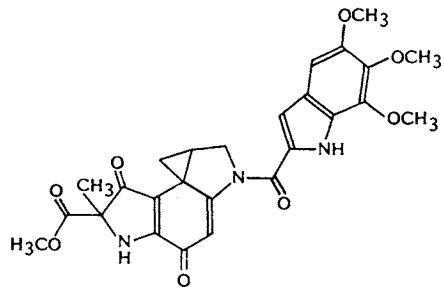
Appearance	Pale yellow powder
$[\alpha]_D^{24}$	+180° (*c* 0.1, MeOH)
HREI-MS	
Calcd for $C_{25}H_{23}N_3O_7$:	477.1534
Found:	477.1522
UV λ_{max}^{MeOH} nm (ε)	235 (sh, 21,000), 316 (16,000), 367 (27,000)
IR *ν* ($CHCl_3$) cm^{-1}	3460, 1714, 1642, 1619, 1517, 1489, 1399, 1389, 1300, 1258, 1215 (sh), 1106
Rf value[a]	0.38
Solubility	
Soluble:	MeOH, $CHCl_3$, EtOAc, $(CH_3)_2CO$, DMSO
Insoluble:	Hexane, water

[a] Merck Art. No. 15647, toluene-acetone (7:3).

Fig. 1. The structures of duocarmycin SA and duocarmycin A.



Duocarmycin SA



Duocarmycin A

cin SA was effective against murine lymphocytic leukemia P388 transplanted in CDF_1 mice, showing a significant increase in life span: ILS 30% at single ip doses of 0.0063 mg/kg. The compound also showed antitumor activity against murine sarcoma 180 in *dd*Y mice: T/C=0.21 at single iv doses of 0.10 mg/kg. The results of our work show that duocarmycin SA is a new antibiotic with high antimicrobial and antitumor potency. Further detailed studies on antitumor spectra and toxicity of duocarmycin SA are in progress and will be reported in due course.

Acknowledgments

The authors are grateful to Dr. T. TAMAOKI and Dr. H. SANO for providing encouragement and helpful suggestions and thank Mrs. MAYUMI YOSHIDA for the NMR spectra and also thank Miss. HISAKO OHBA and Miss. KAZUE HARA for their technical assistances.

MICHIO ICHIMURA†
TATSUHIRO OGAWA†
KEI-ICHI TAKAHASHI†
EIJI KOBAYASHI†
ISAO KAWAMOTO
TOHRU YASUZAWA
ISAMI TAKAHASHI*
HIROFUMI NAKANO

Tokyo Research Laboratories,
Kyowa Hakko Kogyo Co., Ltd.,
Machida, Tokyo, Japan
† Pharmaceutical Research Laboratories,
Kyowa Hakko Kogyo Co., Ltd.,
Nagaizumi, Shizuoka, Japan

(Received March 2, 1990)

References

1) TAKAHASHI, I.; K. TAKAHASHI, M. ICHIMURA, M. MORIMOTO, K. ASANO, I. KAWAMOTO, F. TOMITA & H. NAKANO: Duocarmycin A, a new antitumor antibiotic from *Streptomyces*. J. Antibiotics 41: 1915~1917, 1988
2) 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: 1285~1288, 1988
3) YASUZAWA, T.; T. IIDA, K. MUROI, M. ICHIMURA, K. TAKAHASHI & H. SANO: Structures of duocarmycins, novel antitumor antibiotics produced by *Streptomyces* sp. Chem. Pharm. Bull. 36: 3728~3731, 1988
4) OGAWA, T.; M. ICHIMURA, S. KATSUMATA, M. MORIMOTO & K. TAKAHASHI: New antitumor antibiotics, duocarmycins B_1 and B_2. J. Antibiotics 42: 1299~1301, 1989
5) OHBA, K.; H. WATABE, T. SASAKI, Y. TAKEUCHI, Y. KODAMA, T. NAKAZAWA, H. YAMAMOTO, T. SHOMURA, M. SEZAKI & S. KONDO: Pyrindamycins A and B, new antitumor antibiotics. J. Antibiotics 41: 1515~1519, 1988
6) ISHII, S.; M. NAGASAWA, Y. KARIYA, H. YAMAMOTO, S. INOUYE & S. KONDO: Antitumor activity of pyrindamycins A and B. J. Antibiotics 42: 1713~1717, 1989
7) 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: 902~903, 1980
8) 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 overy cells. Cancer Res. 48: 109~116, 1988