Communication to the Editor

REVEROMYCIN A, A NEW ANTIBIOTIC WHICH INHIBITS THE MITOGENIC ACTIVITY OF EPIDERMAL GROWTH FACTOR

Sir:

In the course of the screening of inhibitors of mitogenic activity induced by epidermal growth factor (EGF)[1] from microbial origin, a new antibiotic, reveromycin A (Fig. 1) was isolated from the culture broth of an actinomycete strain. The producing strain was isolated from a soil sample collected in Gunma Prefecture, Japan and the taxonomic study showed that it belongs to the genus *Streptomyces*. The species is not determined yet. The structure of reveromycin A was elucidated by NMR studies. In this communication, the isolation, structure, and biological activities of reveromycin A is preliminarily reported.

The producing strain was cultured in a 30-liter jar fermenter containing 18-liter of the fermentation medium (glucose 2%, soybean meal 2.5%, soluble starch 1%, meat extract 0.1%, dried yeast 0.4% and NaCl 0.2%, adjusted pH 7.0). The fermentation was carried out at 28°C, 350 rpm stirring speed and 18 liters/minute aeration rate for 72 hours.

The fermentation and the following purification procedure were monitored by the inhibitory activity of [^{3}H]thymidine-uptake into Balb/MK cells stimulated with EGF as described in our previous paper[1]. The culture filtrate was adjusted to pH 10 and extracted with the same volume of EtOAc. In alkaline condition, the antibiotic remained in the aqueous layer. The antibiotic was extracted into the organic layer after the pH of the aqueous layer was adjusted at pH 5. The extract was concentrated and purified by a silica gel column (100% MeOH). The active fractions were combined and further purified

Fig. 1. Structure of reveromycin A.

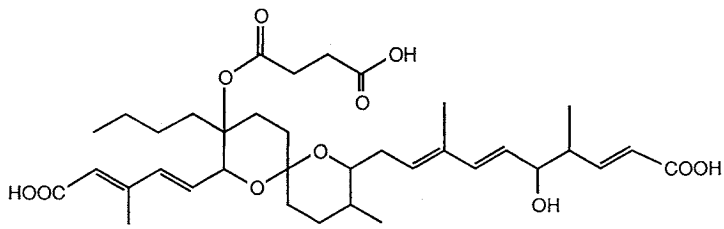


Fig. 2. UV spectra of reveromycin A.

——— MeOH, ----- MeOH - 0.01 N HCl, —·—·— MeOH - 0.01 N NaOH.

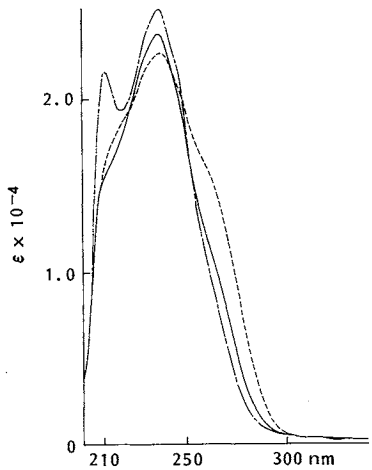


Fig. 3. ^1H NMR of reveromycin A (500 MHz, CD_3OD).

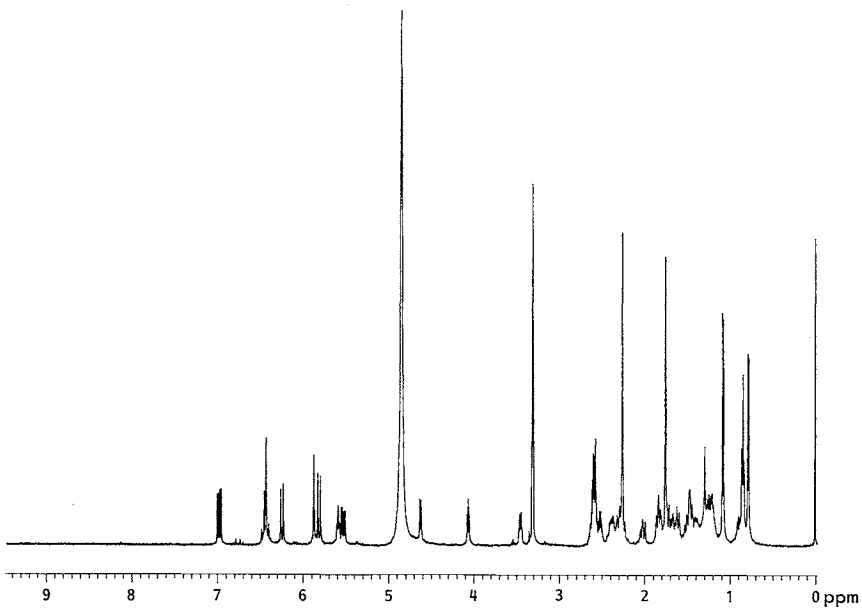
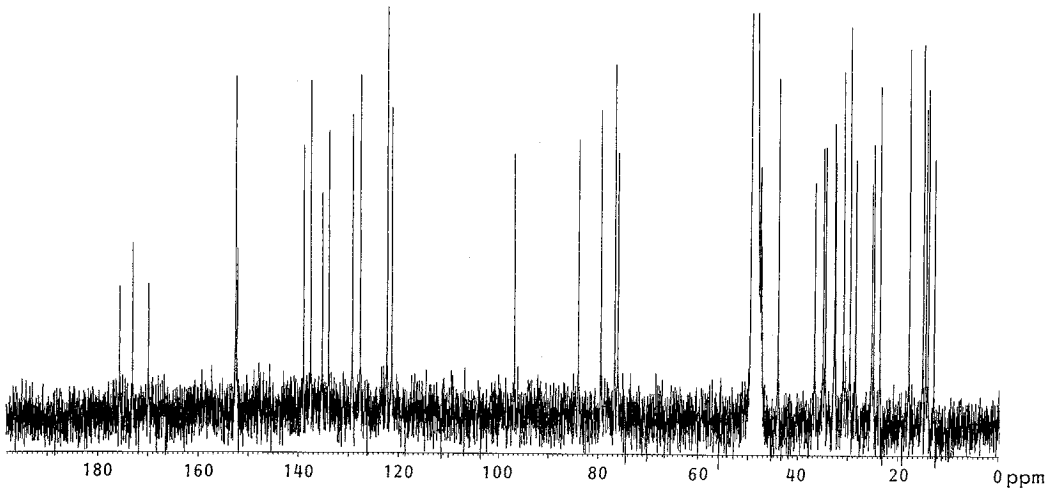


Fig. 4. ^{13}C NMR of reveromycin A (125 MHz, CD_3OD).



by MCI gel (70% MeOH) and Sephadex LH-20 (20% MeOH) column chromatography successively. The antibiotic was finally purified with a reverse phase column (Capcell Pak C_{18}), which was eluted with MeOH-H_2O-1% NH_4OH (18:81:1). Approximately, 100 mg of the antibiotic was obtained from 18-liter fermentation broth.

Reveromycin A is a white amorphous powder with mp 95°C. It is optically active; $[\alpha]_D^{20}$ −115° (*c* 0.1, MeOH). UV absorption maxima in MeOH are 238 (ε 25,300) and 260 nm (sh, ε 12,200) (Fig. 2). HRFAB-MS and elemental analysis indicated the molecular formula $C_{36}H_{52}O_{11}$: found *m/z* 683.3496 $(M+Na)^+$, calcd for $C_{36}H_{52}O_{11}Na$ 683.3586. *Anal* Calcd for $C_{36}H_{52}O_{11}\cdot\frac{1}{2}H_2O$; C 64.57, H 7.92. Found: C 65.45, H 8.06. The ^1H and ^{13}C NMR spectra are shown in Figs. 3 and 4. The structure of the antibiotic was elucidated as shown in Fig. 1 by analyses of the correlation of ^1H-^1H COSY, ^1H-^{13}C COSY and heteronuclear multiple-bond correlation (HMBC) spectra. The presence of three carboxylic acid and a hydroxy group was supported by methylation (CH_2N_2) and acetylation (Ac_2O-pyridine). Reveromycin A has a unique polyketide dicarboxylic acid structure with a spiroketal, a butyl side chain and a succinate moiety. Details of

the structural study will be reported elsewhere.

Reveromycin A inhibited the incorporation of [^{3}H]thymidine into Balb/MK cells stimulated with EGF (IC_{50} about 1 μg/ml). Inhibitors of mitogenic activity induced by EGF is a candidate of the G_0/G_1 arresting reagent in cell cycle. Reveromycin A also blocked the progression of the cell cycle of a virus (ts25, a T-class temperature sensitive mutant of Rous sarcoma virus Prague strain) transformed NRK cells (*src*^{ts}-NRK) from G_1 phase to S phase at the concentration of 5 μg/ml. The transformed morphology of the cell was reversed by the antibiotic at the same concentration.

The antibiotic showed antifungal activity against plant pathogenic fungi at the MIC ranging from 16 to 64 μg/ml. The MIC against *Candida albicans* IFO 1594 was 250 μg/ml. Bacteria tested were not sensitive to the highest concentration tested (250 μg/ml).

Acknowledgments

We are grateful to Mr. Y. ESUMI and Mr. K. TANAKA (Jeol) for MS, and to Dr. J. UZAWA and Ms. T. CHIJIMATSU for NMR measurement. We thank Dr. M. URAMOTO and Ms. M. NISHI for discussion and assistance. This research was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan. HK is a Special Researcher of Basic Science Program supported by Science and Technology Agency, Japan.

HIROYUKI OSADA
HIROYUKI KOSHINO
KIYOSHI ISONO*

Antibiotics Laboratory,
RIKEN (The Institute of Physical and Chemical Research),
Wako, Saitama 351-01, Japan

HIDETOSHI TAKAHASHI
GOSEI KAWANISHI

Research Institute of Life Science,
Snow Brand Milk Products Co., Ltd.,
Ishibashi, Tochigi 329-05, Japan

(Received August 3, 1990)

References

1) OSADA, H.; T. SONODA, H. KUSAKABE & K. ISONO: Epiderstatin, a new inhibitor of the mitogenic activity induced by epidermal growth factor. I. Taxonomy, fermentation, isolation and characterization. J. Antibiotics 42: 1599~1606, 1989