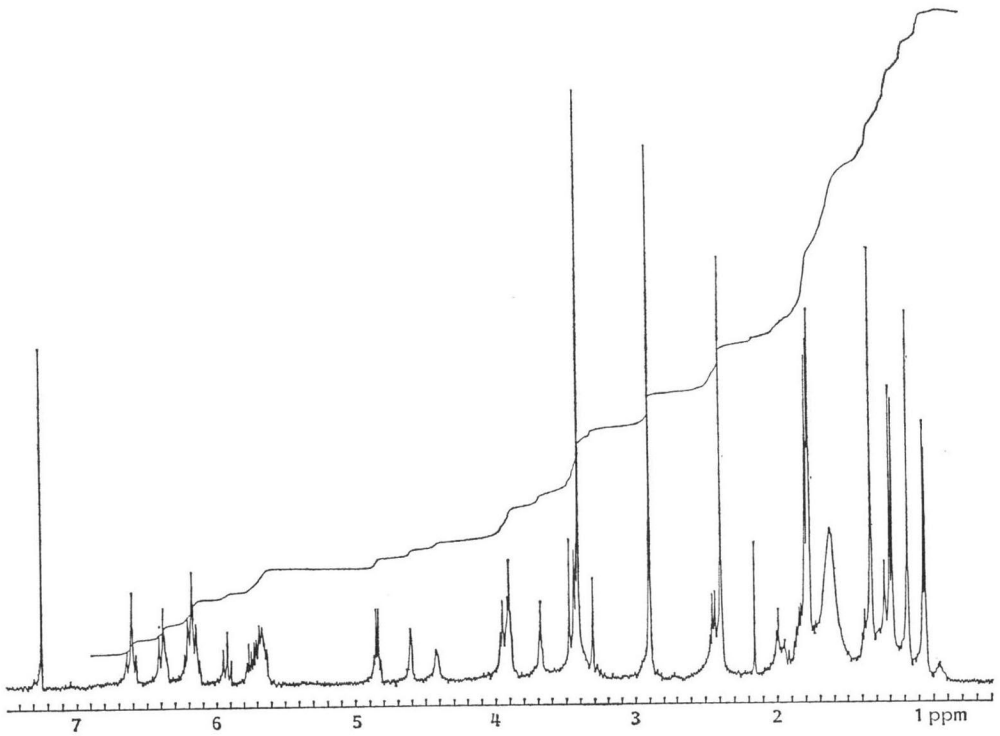
IM8443T SUBSTANCE, AN ANTITUMOR TRIENE β-LACTONE ANTIBIOTIC

Sir:

During the course of screening for new antitumor antibiotics, we found a triene β-lactone antibiotic in the culture broth of *Streptomyces hygroscopicus* IM8443T, which was isolated from soil collected last year in Yamanashi Prefecture. The strain was cultured at 27°C for 4 days on a rotatory shaker in a medium containing oatmeal 2% and yeast extract 0.1%. Flasks were seeded with vegetative inoculum, 2% by volume, grown for 4 days in the same medium. The cultured broth in 2 flasks (1.5 liters×2) was collected and filtered. Antibiotic in the filtrate was adsorbed to Diaion HP20 and eluted with MeOH, and that in the mycelial cake was extracted with MeOH. Both MeOH solutions were combined, concentrated *in vacuo*, and extracted with EtOAc, after pH was adjusted to pH 7.0 with 0.1 N NaOH. The EtOAc layer was concd *in vacuo* and dissolved in $CHCl_3$. The antibiotic was partially purified by adsorption on a column of silica gel, washing with $CHCl_3$ and developing with $CHCl_3$ - MeOH (20: 1). Further purification was accomplished by preparative TLC (silica gel, EtOAc - MeOH, 20: 1) and the active band was extracted with MeOH. Final purification of the MeOH extract was achieved by HPLC (C_{18}-Silica) with a solvent system of CH_3CN - H_2O (45: 55), providing *ca.* 25 mg of IM8443T. Growth-inhibitory activity against L5178Y murine lymphoma and *Bacillus subtilis* was used as the assay for following fermentation and purification studies.

The antibiotic was obtained as a colorless crystalline powder, soluble in $CHCl_3$, EtOAc, EtOH and MeOH, but hardly soluble in H_2O and carbon tetrachloride. The antibiotic, FD-MS: m/z 684 $(M+H)^+$, shows UV λ_{max}^{MeOH} (ε) 231 (20,900), 267 (sh, 19,700), 275 (20,600) and 285 nm (sh, 18,400), and IR $\nu_{max}^{CHCl_3}$ 3330 (OH, NH), 1820 (β-lactone), 1690 (amide I), 1630 (amide II) and 995 (triene) cm^{-1}. ^1H NMR in $CDCl_3$ (400 MHz) showed chemical shifts: $-CH_3\times4$ (0.95~1.35 ppm), $\underset{|}{=C}-CH_3\times2$ (1.80 ppm), $-CH_3\times1$ (2.40 ppm), $N-CH_3\times1$ (2.90 ppm), $O-CH_3\times1$ (3.45 ppm), and $\underset{|}{=CH}\times10$ and

Fig. 1. ^1H NMR spectrum of IM8443T substance ($CDCl_3$, 400 MHz).



NH×1 (5.6~6.7 ppm) (Fig. 1). The physicochemical properties of IM8443T suggest it resembles oxazolomycin[1]. The difference in molecular weights of the two substances, 28, suggests that two methyl groups of the former are replaced by two hydrogen groups in the latter. The 13′-H signal of the oxazole ring of oxazolomycin is not observed in ^{1}H NMR of IM8443T, suggesting that 13′-H is replaced by CH_3 in IM8443T. Recently curromycin B, a dimethyl derivative of oxazolomycin, was found in Prof. ŌTAKE's laboratory[2]. IM8443T was identical with curromycin B by ^{1}H NMR and molecular weight.

IM8443T inhibited *in vitro* growth of murine tumor cells: L5178Y, L1210 and P388. The IC_{50} was 0.01~0.1 μg/ml. The antibiotic also displayed inhibitory activity against Gram-positive bacteria. Typical MIC values were 50 μg/ml for *Staphylococcus aureus* IAM1011, 20 μg/ml for *Micrococcus luteus* IAM1056 (agar dilution method); and 5 μg/ml for *Bacillus subtilis* PCI219, *B. cereus* T and *Corynebacterium xerosis* (disc agar method). No inhibitory activity was observed at an antibiotic concentration of 100 μg/ml against Gram-negative organisms, *Mycobacterium* and fungi.

Acknowledgments

The authors express their deep thanks to Dr. HAMAO UMEZAWA, Institute of Microbial Chemistry, Tokyo, for his kind advice and direction. This work was partly supported by Grant-in-Aid for cancer research from the Ministry of Education, Science and Culture, Japan.

TAKAYOSHI OKABE
FUJIO ISONO
MASAMI KASHIWAGI
MASAKO TAKAHASHI
TOSHIO NISHIMURA
HIDEO SUZUKI
NOBUO TANAKA

Institute of Applied Microbiology,
University of Tokyo
Bunkyo-ku, Tokyo, 113 Japan

(Received April 3, 1985)

References

1) MORI, T.; K. TAKAHASHI, M. KASHIWABARA, D. UEMURA, C. KATAYAMA, S. IWADARE, Y. SHIZURI, R. MITOMO, F. NAKANO & A. MATSUZAKI: Structure of oxazolomycin, a novel β-lactone antibiotic. Tetrahedron Lett. 26: 1073~1076, 1985

2) OGURA, M.; H. NAKAYAMA, A. SHIMAZU, K. FURIHATA, H. SETO & N. ŌTAKE: Studies on the new antibiotic curromycins A and B. The 238th Scientific Meeting of Japan Antibiotics Research Association. Mar. 27, 1985