Communications to the Editor

ENOPEPTIN A, A NOVEL DEPSIPEPTIDE ANTIBIOTIC WITH ANTI-BACTERIOPHAGE ACTIVITY

Sir:

Using actinophage B1 and *Streptomyces griseus* as a convenient prescreening[1] for antiviral substances, a new antibiotic, enopeptin A was isolated from a culture broth of *Streptomyces* sp. RK-1051. Strain RK-1051 was isolated from a soil sample collected in Tsuruoka city, Yamagata Prefecture, Japan, and deposited at the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, under the accession No. FERM P-11624.

The strain was inoculated into a 500-ml cylindrical flask containing 70 ml of a seed medium (glucose 2%, soybean meal 2.5%, soluble starch 1%, meat extract 0.1%, dried yeast 0.4% and NaCl 0.2%, adjusted at pH 7.0) and cultured for 48 hours at 28°C on a rotary shaker (250 rpm). The culture was transferred to a 30-liter jar fermenter containing 18 liters of the same medium and fermentation was carried out for 144 hours at 28°C. The antibiotic potency of 150 μg/ml was obtained after 144-hour fermentation.

The culture filtrate was extracted with EtOAc, which was then concentrated *in vacuo*. The resulting oily material was suspended in a small volume of MeOH, to which an excess volume of hexane was added, affording a yellowish orange precipitate. The precipitate was dissolved in $CHCl_3$ and applied onto a silica gel chromatography column equilibrated with $CHCl_3$ - MeOH (20 : 1). Enopeptin A was eluted by the solvent $CHCl_3$ - MeOH (10 : 1). The active fractions were combined, concentrated, and applied onto a reverse-phase column (Senshu ODS 1020), which was eluted with stepwise gradient of MeOH (60, 80, and 100%). Enopeptin A was eluted with 80% MeOH, from which a yellowish orange crystalline powder was precipitated. Finally, the crystalline powder was collected and dried to yield 25 mg of pure enopeptin A.

Enopeptin A is a yellowish orange crystalline powder with mp 210°C (dec) and optical rotation $[\alpha]_D^{20}$ +270° (*c* 0.45, $CHCl_3$). It is soluble in $CHCl_3$, DMSO, and EtOAc, slightly soluble in MeOH and EtOH, but insoluble in hexane and water. Elemental analysis gave C 60.17, H 6.24, N 10.44. Calcd for $C_{47}H_{57}N_7O_{11} \cdot 2H_2O$: C 60.58, H 6.55, N 10.53. As SI-MS gave two molecular ion peaks, 896 $(M+H)^+$ and 934 $(M+K)^+$, the molecular formula was established to be $C_{47}H_{57}N_7O_{11}$.

Enopeptin A has a characteristic UV spectrum as shown in Fig. 2. UV $\lambda_{max}^{10\%\,DMSO}$ nm (ε) 258 (14,300), 375 (51,000), 393 (45,600). IR, ^1H NMR, and ^{13}C NMR spectra are shown in Figs. 3, 4, and 5,

Fig. 2. UV absorption spectrum of enopeptin A (in 10% DMSO).

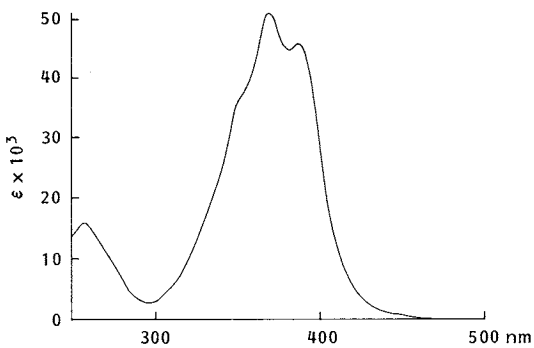


Fig. 1. Structure of enopeptin A.

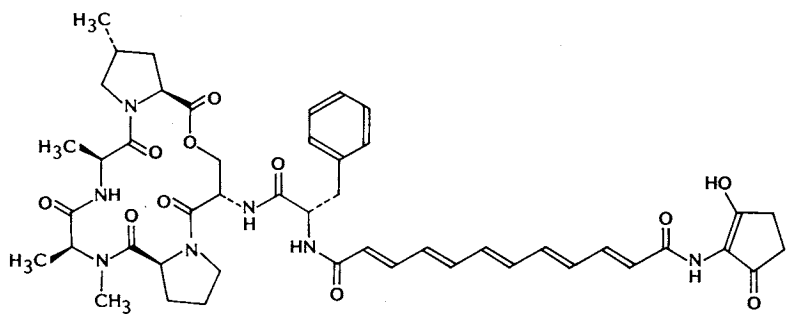


Fig. 3. IR spectrum of enopeptin A (in KBr).

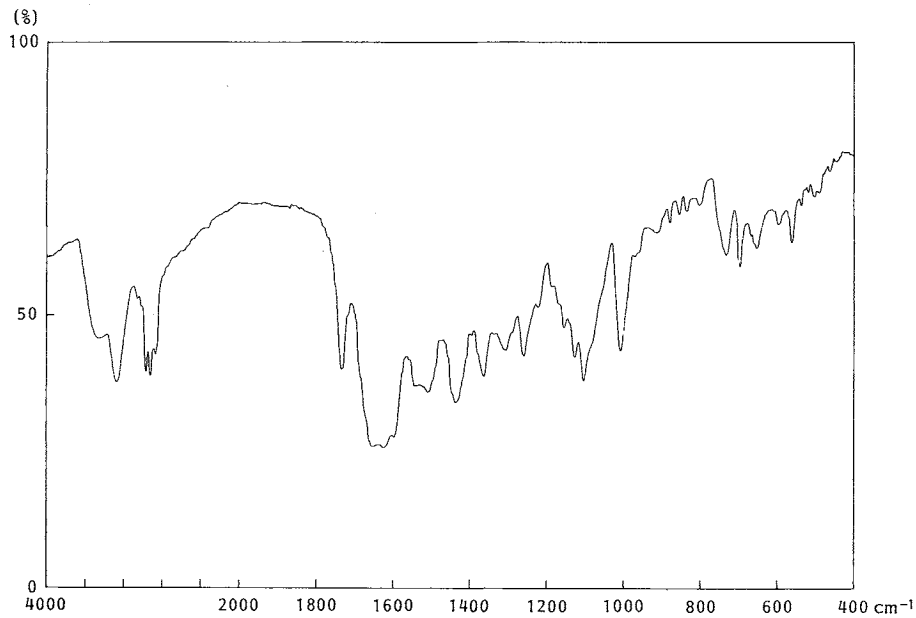
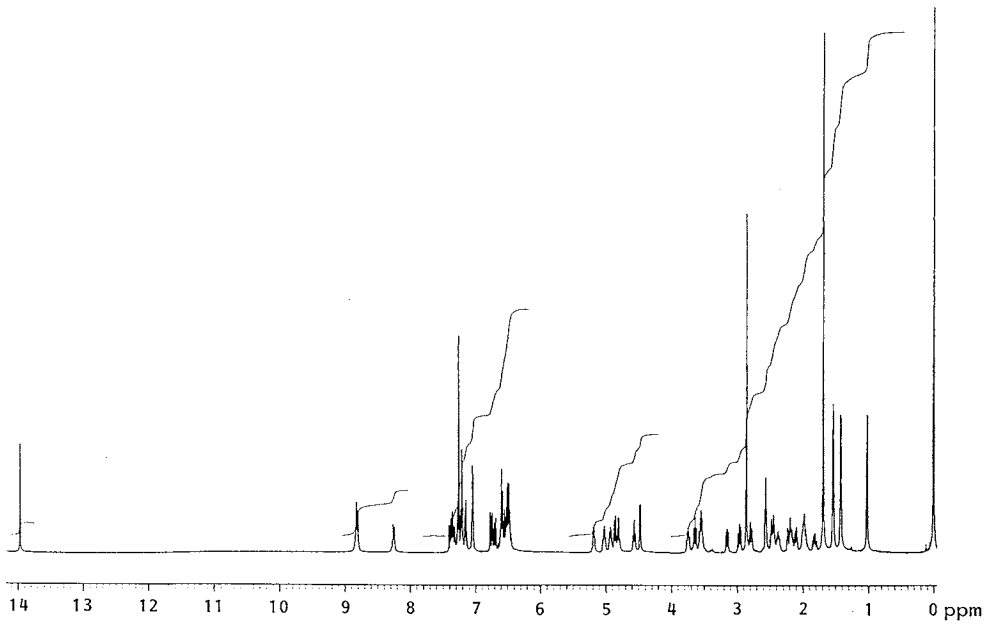


Fig. 4. ^1H NMR spectrum of enopeptin A (500 MHz, in $CDCl_3$).



respectively. Amino acid analysis of the acid hydrolysis of enopeptin A gave alanine, proline, phenylalanine, serine, and two unusual amino acids, which were deduced to be *N*-methylalanine and 4-methylproline from NMR analysis of enopeptin A. Transesterification of enopeptin A in MeOH-NaOH gave a ring-opened methyl ester (*m/z* 950, $(M+Na)^+$).

Enopeptin A has a novel structure consisting of a cyclic depsipeptide, a pentaene dicarboxylic acid,

Fig. 5. ^{13}C NMR spectrum of enopeptin A (100 MHz, in $CDCl_3$).

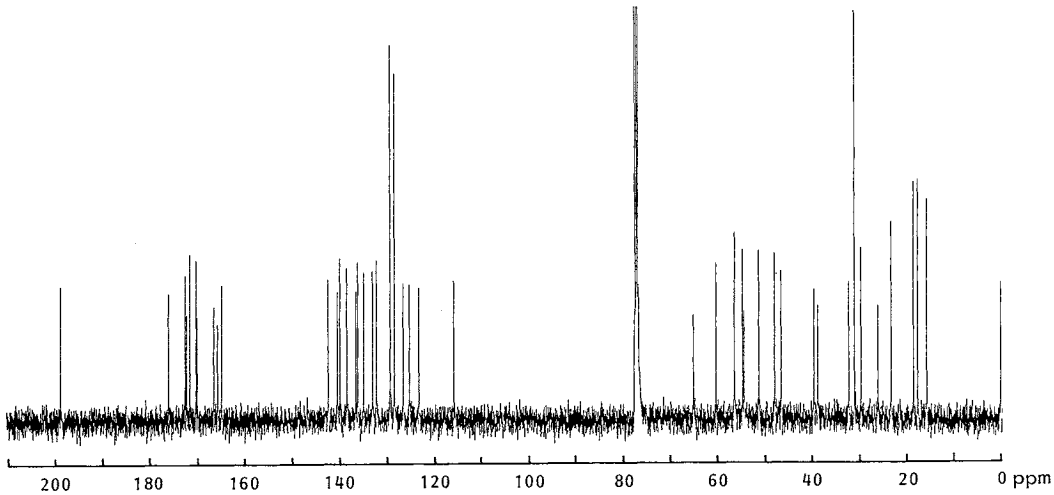


Table 1. Antimicrobial spectrum of enopeptin A.

Organisms tested[a]	MIC (μg/ml)[b]
Staphylococcus aureus FDA 209P JC-1	12.5
S. aureus Smith	25
S. aureus JS-1 (MRSA)	25
Streptococcus faecalis 1373	25
Xanthomonas campestris pv. citri	>200
Escherichia coli JE1011	>200
E. coli BE 1186	200
E. coli HO141	200
Pseudomonas aeruginosa IFO 13130	>200
P. aeruginosa L-form	200
Pyricularia oryzae IFO 5994	>200
Botryotinia fuckeliana IFO 5365	>200
Alternaria mali IFO 8984	>200

[a] Modified Mueller-Hinton agar (Nissui) was used for bacterial culture except *Xanthomonas campestris*. Potato-sucrose agar was used for *X. campestris* and fungi.

[b] Determined by agar dilution method.

and a 2-amino-3-hydroxycyclopent-2-enone (Fig. 1). The structure determination will be a subject of a separate publication[2]. Antibiotic A54556[3] and virustomycin[4] have a part of the moieties of enopeptin A, however, the molecular formula and the total structure are clearly different.

When the antibacteriophage activity was measured by the modified paper disk-agar plate method[1], enopeptin A inhibited the plaque formation of bacteriophage B at the concentration of 5 μg/disk (MIC). As shown in Table 1, it showed antimicrobial activity against Gram-positive bacteria including a methicillin-resistant *Staphylococcus aureus* and Gram-negative mutants which are defective in cell membrane (*Escherichia coli* BE 1186, *E. coli* HO141 and *Pseudomonas aeruginosa* L-form N-10). The antibiotic was not inhibitory to fungi at the concentration tested.

Acute toxicity of enopeptin A to mice was low. LD_{50} was approximately 200 mg/kg (ICR mice, ip).

Acknowledgments

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