COMMUNICATIONS TO THE EDITOR

A New Cyclic Lipopeptide Antibiotic, Enamidonin

Sir:

We have been screening microbial fermentation for inhibitors of mitogenic activity of epidermal growth factor[1,2]. During the screening, a new lipopeptide antibiotic, enamidonin (Fig. 1) which shows detransforming activity of a rat cell line transformed with a temperature sensitive Rous sarcoma virus (*src*^{ts}-NRK cells), has been isolated from a culture broth of *Streptomyces* sp. 91-75. The producing strain was isolated from a soil sample collected in Imaichi city, Tochigi prefecture and deposited at the National Institute of Bioscience and Human-Technology under the accession number FERM P-14190.

The strain was cultured at 28°C for 72 hours in 500 ml shaking flasks containing 70 ml of a medium composed of glucose 2%, soluble starch 1%, meat extract 0.1%, dried yeast 0.4%, soybean flour 2.5%, sodium chloride 0.2% and dipotassium hydrogen phosphate 0.005%, adjusted to pH 7.0 before sterilization. A culture broth (5 liters) was filtered and the mycelial cake was extracted with 70% aqueous acetone. After removal of acetone, the aqueous residue was extracted twice with 5 liters of ethyl acetate. The extracts were combined and concentrated *in vacuo* to yield 0.3 g of a syrup. The syrupy residue was applied to a silica gel column (6 × 90 cm) equilibrated with chloroform. The column was eluted stepwise with chloroform - methanol (50 : 1, 10 : 1, 5 : 1 and 1 : 1). Active fractions eluted with solvents 5 : 1 to 1 : 1 were combined and concentrated *in vacuo* to yield 60 mg of a white powder. This was further purified by chromatography on a Sephadex LH-20 column with chloroform - methanol (1 : 1). A white powder (7.2 mg)

Fig. 1. Structure of enamidonin.

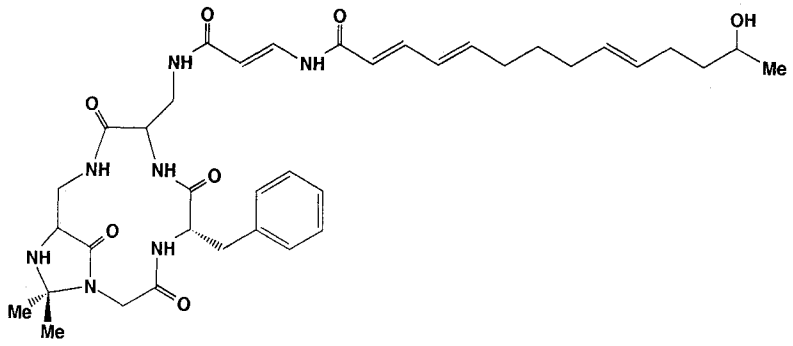


Fig. 2. ^{1}H NMR of enamidonin in DMSO-d_6, 600 MHz (ppm from TMS as an internal standard).

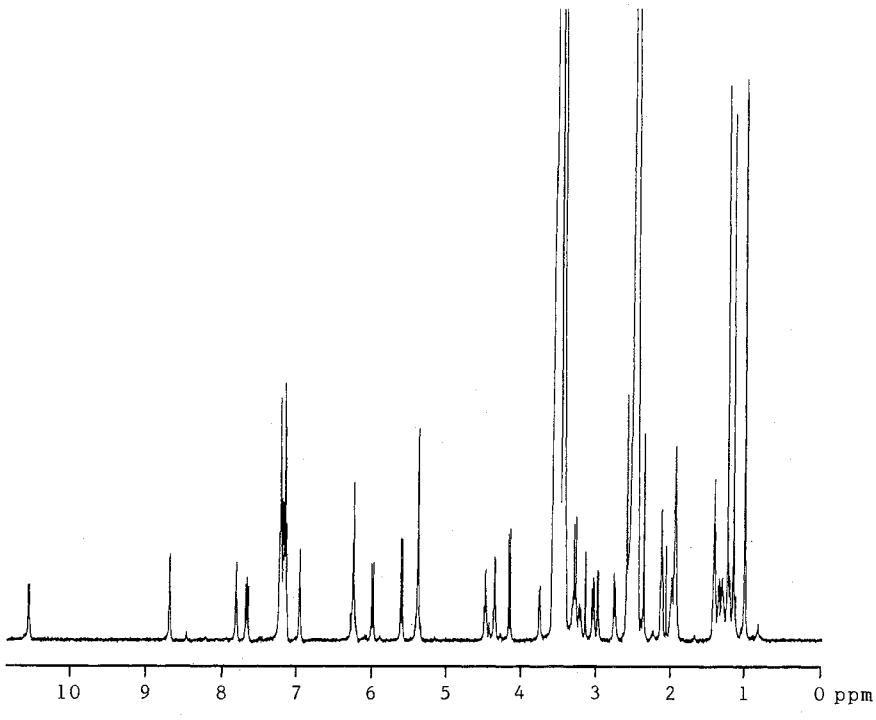
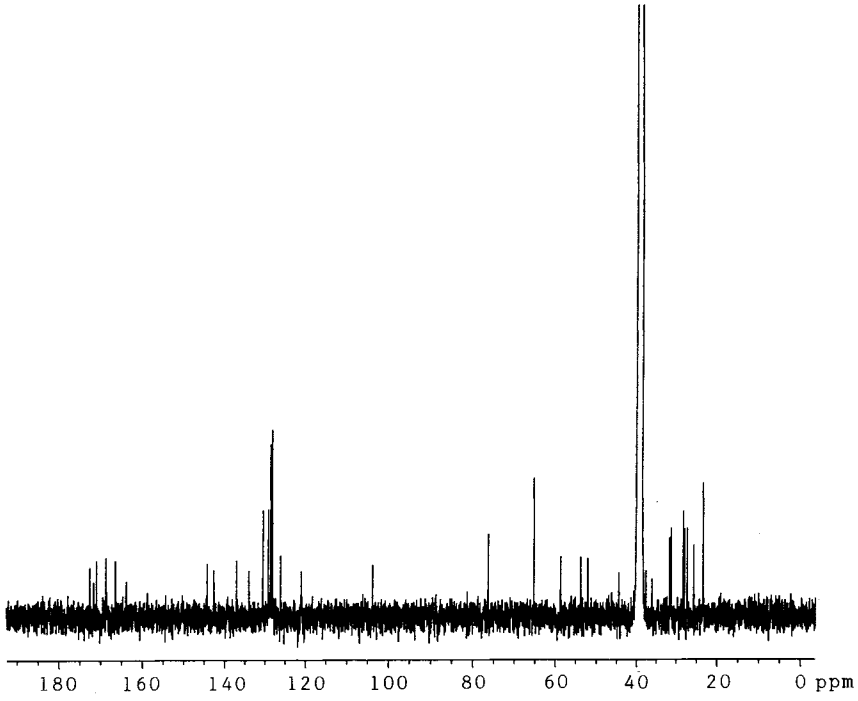


Fig. 3. ^{13}C NMR of enamidonin in DMSO-d_6, 100 MHz (ppm, $(CD_3)_2SO$ as an internal standard at 39.5 ppm).

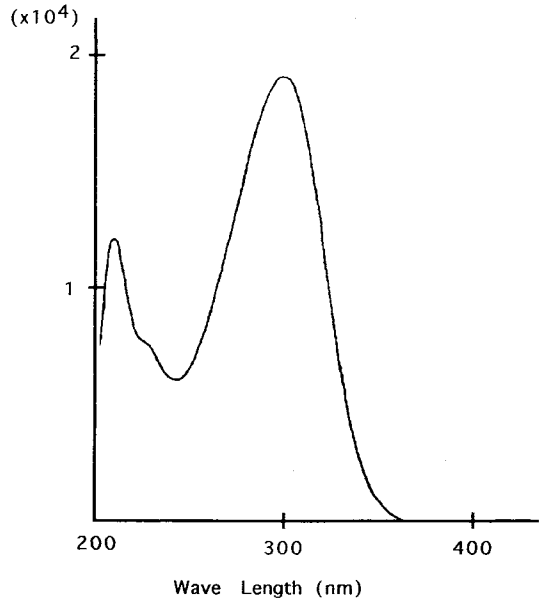


was obtained by concentration of active fractions. Final purification was achieved by preparative HPLC on a column (20 × 250 mm), Capcell Pak C_{18} (Shiseido, Tokyo, Japan) with 70% methanol. Active fractions were combined and concentrated *in vacuo* to remove methanol. The resulting aqueous suspension was lyophilized, affording 2.3 mg of enamidonin as a white powder.

The antibiotic decomposed at 180~220°C. It was optically active with $[\alpha]_D^{20}$ +20° (*c* 0.03, methanol). It was soluble in dimethylsulfoxide (DMSO), acetone and methanol but sparingly soluble in chloroform. It was insoluble in water or hexane. Enamidonin gave positive reactions to ninhydrin and anisaldehyde-H_2SO_4 tests. Amino acid analysis indicated the presence of glycine, phenylalanine and 2,3-diaminopropionic acid. Phenylalanine was determined to be *L* by HPLC analysis of the (+)-1-(9-fluorenyl)ethyl chloroformate (FLEC) derivative[3]. Secondary ionization mass spectrometry (SI-MS) gave $(M+H)^+$ *m/z* 706 and $(M+Na)^+$ *m/z* 728. The molecular formula of enamidonin, $C_{37}H_{51}N_7O_7$ was determined by high resolution fast atom bombardment HRFAB-MS which gave MH^+ *m/z* 706.3972 (Calcd for $C_{37}H_{52}N_7O_7$, MH^+ 706.3928). The molecular formula is consistent with the ^1H and ^{13}C NMR data shown in Figs. 2 and 3, respectively. Because there are two pairs of equivalent phenyl carbons, 35 carbon signals were observed in Fig. 3. The UV absorption spectrum in MeOH showed the following maxima; λ_{max} nm (ε) in MeOH; 223 (7,760) and 302 (18,890) (Fig. 4). The IR spectrum is shown in Fig. 5.

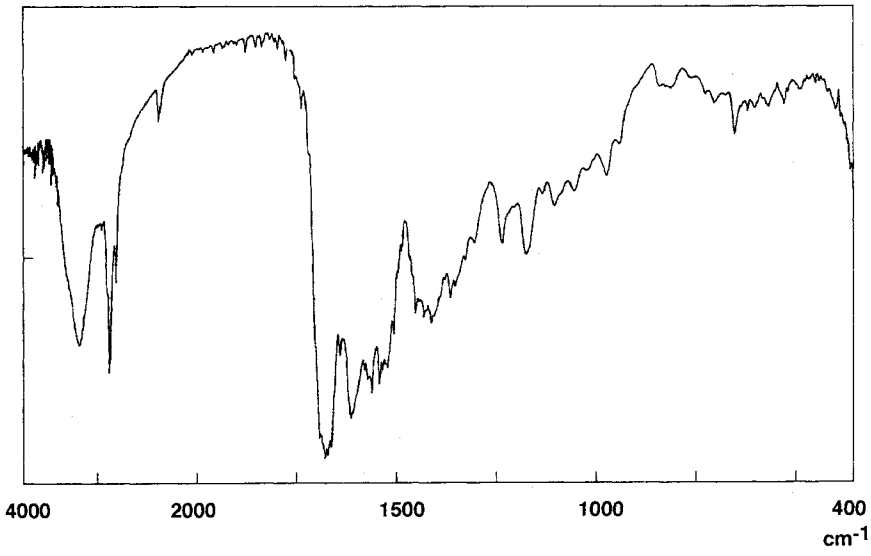
All amino acid components of enamidonin were supported by ^{13}C and ^1H NMR data. By detailed 2D NMR experiments, all ^1H and ^{13}C signals and the presence of *N,N*-isopropylidene, (*E*)-3-aminopropenoic acid and (2*E*,4*E*,9*E*)-13-hydroxytetradeca-2,4,9-trienoic acid moieties were revealed. Connectivities of the partial structures and four amino acid residues were established by HMBC and NOE data. The presence of five amide NH groups and one imine NH group were confirmed by the ^{15}N-^1H HMQC technique. Details of the structural determination will be reported in a separate paper.

Fig. 4. UV spectrum of enamidonin in MeOH.



Enamidonin belongs to a group of cyclic lipopeptides. Recently, several cyclic lipopeptides have been isolated, for example, enopeptin[4], pneumocandin[5] and aselacin[6]. These lipopeptides are cyclic peptides with a long acyl side chain connected via an amide bond like enamidonin, however, they are different from enamidonin in their

Fig. 5. IR spectrum of enamidonin in KBr.



molecular formula and biological activity. Enamidonin inhibited the EGF dependent uptake of [^{3}H]thymidine into Balb/MK cells at the concentration of 10 μg/ml (IC_{50}). The antibiotic also reversed the transformed morphology of *src*^{ts}-NRK to the normal flat morphology at the concentration of 10 μg/ml (ED_{50}). Enamidonin was not inhibitory to fungi and bacteria tested on a conventional paper disk-agar method at the concentration 4 μg/disk. Detailed biological activity and the mechanism of action studies of enamidonin are in progress.

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