PYRIZINOSTATIN: A NEW INHIBITOR OF PYROGLUTAMYL PEPTIDASE

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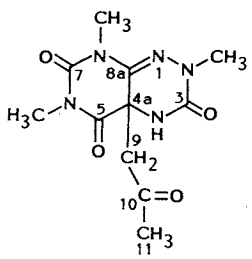
While screening the culture filtrate of microorganisms for specific inhibitors against various kinds of enzymes, we found a variety of new compounds that specifically inhibit the target enzymes[1~3]. Among such compounds was included benarthin[4,5], an inhibitor of pyroglutamyl peptidase (PG-peptidase). We have further continued screening for inhibitors of PG-peptidase, and discovered another new inhibitor, which we named pyrizinostatin, from culture filtrate of the microbial strain SA2289, which had been isolated from a marine soil and confirmed to belong to the genus *Streptomyces*. The structure of pyrizinostatin was elucidated by NMR spectral analysis and X-ray analysis, and was determined as 2,4,4a,8-tetrahydro-2,6,8-trimethyl-4a-(2-oxopropyl)pyrimido[5,4-*e*]-1,2,4-triazine-3,5,7(6*H*)-trione (Fig. 1).

The activity of PG-peptidase was measured according to the method of EXTERKATE[6], as reported previously[4], in order to determine a concentration of the inhibitor required for 50% inhibition (IC_{50}).

Pyrizinostatin was produced by shaken culture of SA2289 in a medium containing soluble starch 1.0%, K_2HPO_4 0.2%, $MgSO_4 \cdot 7H_2O$ 0.1%, $(NH_4)_2SO_4$ 0.2%, yeast extract 0.1%, $FeSO_4 \cdot 7H_2O$ 0.0001%, $MnCl_2 \cdot 4H_2O$ 0.0001%, $ZnSO_4 \cdot 7H_2O$ 0.0001%, $CaCO_3$ 0.2% and Jamarin S (Jamarin Laboratory) 0.01%, adjusted to pH 7.2 with 5N NaOH before sterilization.

Fig. 1. Structure of pyrizinostatin.



The flow diagram for the isolation is shown in Scheme 1. The broth was harvested after 96 hours and filtered to give broth filtrate (14 liters, pH 5.9). The inhibitor in broth filtrate was absorbed on Diaion HP-20, inhibitor was eluted with 0~75% MeOH. The solution containing inhibitor was absorbed on a column of Sepabeads SP-206 and eluted with 0~90% MeOH. The eluate solution containing active compound was concentrated to give a crude powder.

The crude powder was subjected to centrifugal partition chromatography (solvent system; BuOH - AcOH - H_2O and $CHCl_3$ - MeOH - H_2O). The fractions containing pyrizinostatin were concentrated to yield a yellow powder. Further purification of the yellow powder was effected by Sephadex LH-20 with MeOH. The fractions containing pyrizinostatin were concentrated, and crystallization from MeOH gave pure pyrizinostatin (40.2 mg) as colorless crystals.

Physico-chemical properties of pyrizinostatin are summarized in Table 1. The molecular formula of pyrizinostatin was determined as $C_{11}H_{15}N_5O_4$ by HRFAB-MS and elemental analysis. The substance gave positive color reactions to molybdophosphoric acid-sulfalic acid and GREIG-LEABACK[7] reagents, and negative to ninhydrin reagent. Pyrizinostatin is

Scheme 1. Isolation of pyrizinostatin.

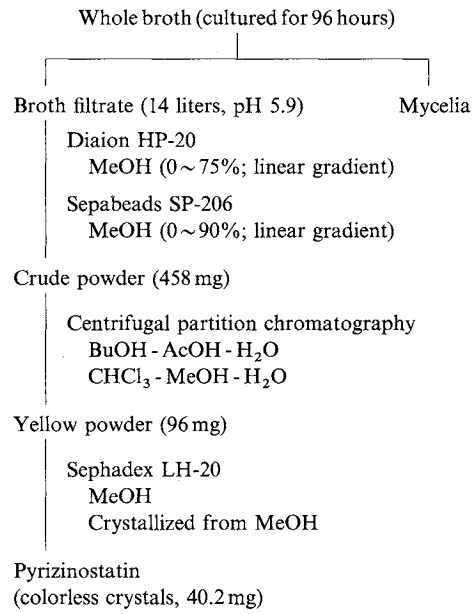


Table 1. Physico-chemical properties of pyrizinostatin.

Appearance	Colorless crystal
MP	188~190°C
$[\alpha]_D^{24}$	−15.6° (*c* 1.0, MeOH)
Molecular formula	$C_{11}H_{15}N_5O_4$
Elemental analysis	
Calcd for $C_{11}H_{15}N_5O_4$:	C 46.92, H 5.33, N 24.89
Found:	C 46.54, H 5.60, N 24.64
HRFAB-MS (*m/z*)	
Calcd for $C_{11}H_{16}N_5O_4$:	282.1208
Found:	282.1205
UV spectrometry	UV λ_{max}^{MeOH} nm (ε), 280 (4,600)
Color reaction	Mo-H_2SO_4, GREIG-LEABACK
Solubility	Soluble; H_2O, $CHCl_3$, MeOH, DMSO
Rf[a]	0.72

[a] On silica gel TLC plate (Merck Art. No. 5715) with BuOH-AcOH-H_2O (4:1:2) as eluent.

Table 2. ^{13}C (100 MHz) and ^1H (400 MHz) NMR data of pyrizinostatin in $CDCl_3$.

Position	^{13}C	M	^1H (*J*=Hz)
N2-CH_3	37.0	q	3.31
3	151.5	s	—
N4-H	—	—	5.75
4a	54.9	s	—
5	166.2	s	—
N6-CH_3	28.8	q	3.27
7	149.8	s	—
N8-CH_3	30.4	q	3.34
8a	138.6	s	—
9	49.6	t	2.95 (16.0)
	—	—	3.24 (16.0)
10	202.7	s	—
11	30.8	q	2.13

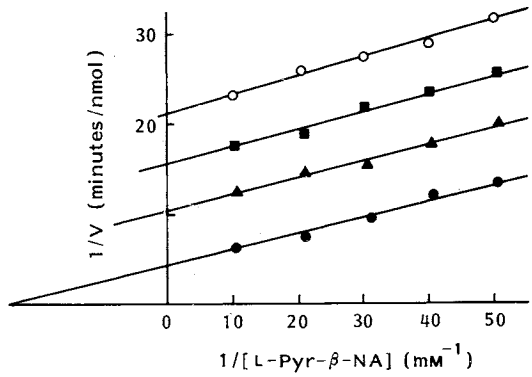
Chemical shifts in ppm from TMS.
M: Multiplicity.

soluble in H_2O, $CHCl_3$, MeOH and DMSO, but insoluble in EtOAc, ether and hexane. The ^{13}C and ^1H NMR data for pyrizinostatin are summarized in Table 2.

The inhibitory activities of pyrizinostatin showed an IC_{50} value of 1.8 μg/ml against PG-peptidase. As shown in Fig. 2, the inhibition of pyrizinostatin against PG-peptidase is noncompetitive. Pyrizinostatin inhibited the growth of *Shigella sonnei* JS11764 and *Proteus vulgaris* OX19 at 100 μg/ml, but had no antimicrobial activity against other bacteria and fungi. Pyrizinostatin had a low toxicity; no deaths occurred after its intravenous injection of 100 mg/kg to mice.

Fig. 2. Lineweaver-Burk plot of inhibition of PG-peptidase by pyrizinostatin.

Pyrizinostatin: ○; 4 μg/ml, ■; 3 μg/ml, ▲; 2 μg/ml, ●; 0 μg/ml. $Km = 4.34 \times 10^{-5}$ M.



Acknowledgments

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