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PIPERASTATIN B: A NEW SELECTIVE SERINE CARBOXYPEPTIDASE INHIBITOR FROM *STREPTOMYCES LAVENDOFOLIAE* MJ908-WF13

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Piperastatin B, a new inhibitor of serine carboxypeptidase was purified from a culture broth of *Streptomyces lavendofoliae* MJ908-WF13 as a minor component by monitoring its inhibitory activity against carboxypeptidase Y (CP-Y). Its structure was determined to be *N*-formyl-Val-Thr-Leu-Val-Pip-Leu-Pip (pip: piperazic acid, hexahydropyridadine-3-carboxylic acid). Piperastatin B is a highly specific competitive inhibitor of CP-Y (K_i = 55 nM) with little effect on related enzymes and resembles the major component, piperastatin A, in these respects.

Keywords: Serine carboxypeptidase, carboxypeptidase Y (EC 3.4.16.1), enzyme inhibitor, peptide, natural compound

INTRODUCTION

Serine carboxypeptidases have different tissue specificities and substrate specificities[1] when compared with metallo carboxypeptidases, such as carboxypeptidase A (CP-A), CP-B[2] or CP-N.[3] Carboxypeptidase Y,[1] gene product of KEX1 of yeast,[4] CP-W of wheat[5] or platelet deamidase of human[6] are also serine carboxypeptidases, and CP-Y-like kininase was recently reported to contribute to the degradation of

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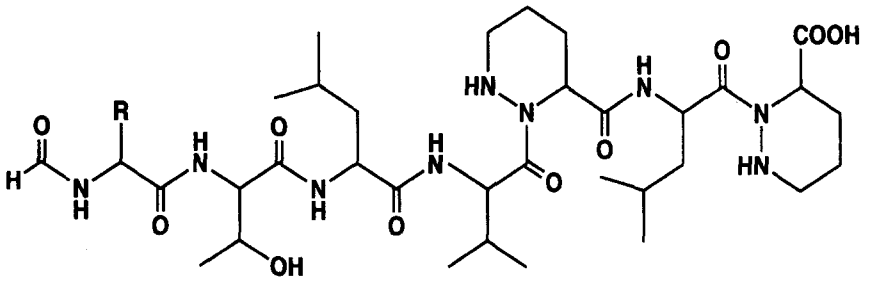
ABBREVIATIONS: Boc, *t*-butyloxycarbonyl; Bz, benzoyl; Suc, succinyl; Z, carbobenzoxy; βNA, β-naphthylamide; CPC, centrifugal partition chromatography; MW, molecular weight, FAB-MS, fast atom bombardment mass spectrometry; HRFAB-MS, high resolution FAB-MS; DEPT, distortionless enhancement by polarization transfer; COSY, correlated spectroscopy; HSQC, heteronuclear single quantum coherence; HMBC, heteronuclear multiple bond correlation.



A : R = $CH(CH_3)CH_2CH_3$
B : R = $CH(CH_3)_2$

FIGURE 1 Structures of piperastatins A and B.

bradykinin in rat urine[7] and to be a candidate for having an important role in the hypertensive pathway.[8] In order to understand the physiological roles of these enzymes by finding a useful tool for biochemical, cell biological or pharmaceutical studies on them, we screened inhibitors in microbial products[9] and discovered piperastatin A,[10,11] a new enzyme inhibitor against CP-Y as a major component in a culture broth of *Streptomyces lavendofoliae* MJ908-WF13.

In this communication we report the isolation, structure determination and enzymatic inhibitory activities of a new inhibitor of serine carboxypeptidase, piperastatin B (Figure 1), which occurs as a minor component from the same microorganism.

MATERIALS AND METHODS

Chemicals

Chemicals employed were as follows: Activated carbon from Wako Pure Chemical Industries Ltd. Osaka, Japan; TLC-plate Silica gel F254 (0.25 mm thickness) from E. Merck, Darmstadt, FRG; Sephadex LH20 from Pharmacia Fine Chemicals AB, Uppsala, Sweden; YMC gel ODS-A from Yamamura Chemical Laboratories Co. Ltd., Kyoto, Japan; Capcellpak C18 UG120 reversed-phase column (4.6 × 150 mm) and Capcellpak C18 SG120 reversed-phase column (20 × 250 mm) from Shiseido Co., Tokyo, Japan; Shodex Asahipak ODP-50

(21.5 × 300 mm), Showa Denko Co. Ltd., Tokyo, Japan; *p*-nitrophenyl acetate (PNP acetate), benzoyl-glycyl-L-phenylalanine (Bz-Gly-Phe), L-leucine-β-naphthylamide (Leu-βNA) from Sigma Chem. Ltd., Saint Louis, USA; benzoyl-glycyl-L-lysine (Bz-Gly-Lys), benzoyl-glycyl-L-histidyl-L-leucine (Bz-Gly-His-Leu), *t*-butyloxycarbonyl-L-valyl-L-prolyl-L-arginine 4-methyl-coumaryl-7-amide (Boc-V-P-R-MCA), succinyl-L-leucyl-L-leucyl-L-valyl-L-tyrosine 4-methyl-coumaryl-7-amide (Suc-L-L-V-Y-MCA) from Peptide Institute Inc., Minoh-shi, Japan; glycyl-L-arginine-β-naphthylamide (Gly-Arg-βNA), L-lysyl-L-alanine-β-naphthylamide (Lys-Ala-βNA), glycyl-L-proline-β-naphthylamide (Gly-Pro-βNA), carbobenzoxy-L-arginyl-L-arginine-β-naphthylamide (Z-Arg-Arg-βNA), benzoyl L-phenylalanyl-L-valyl-L-arginine-4-methoxy-β-naphthylamide (Bz-Phe-Val-Arg-4-methoxy-βNA), carbobenzoxy-glycyl-L-proline-β-naphthylamide (Z-Gly-Pro-βNA) from Bachem Freinchemikalien AG, Budendorf, Switzerland; MGPA from Calbiochem. Co., La Jolla, USA. All other chemicals were of analytical grade.

Enzymes

Carboxypeptidase Y (CP-Y, EC 3.4.16.1) from yeast was obtained from Oriental Yeast Co. Ltd., Japan. Carboxypeptidase A (CP-A) type I from bovine pancreas, thrombin from human plasma, α-chymotrypsin type II from bovine pancreas and cathepsin B from bovine spleen were purchased from Sigma Chem. Ltd., Saint Louis, USA. Carboxypeptidase B (CP-B) from porcine pancreas, aminopeptidase N (AP-N) from hog kidney, leucine aminopeptidase (Leu-AP) from hog kidney and esterase from hog liver were purchased from Boehlinger Mannheim GmbH, FRG. Lipase from hog pancreas was obtained from Nutritional Biochem Co., USA. Carboxypeptidase N (CP-N) from human plasma, angiotensin converting enzyme (ACE), dipeptidylaminopeptidase I (DPP-I) from rat spleen, DPP-II from rat spleen, DPP-IV from rat kidney and prolylendopeptidase (PEP) from hog kidney were partially purified as described in a previous paper.[10]

Isolation of Piperastatin B

The purification procedure of piperastatin B is shown in Figure 2. After 112 h incubation at 30°C, the cultured broth was separated from mycelium by filtration. The culture filtrate (15 litres) was applied to an activated carbon column (1.5 litres). The column was washed with water and was eluted with 70% aqueous 1-propanol (pH 7.0). The active fractions were concentrated and lyophilized to give a crude powder (15.0 g). The crude powder was dissolved in water, and subjected to a YMC-gel ODS-A column (250 ml) and then eluted with a linear gradient from

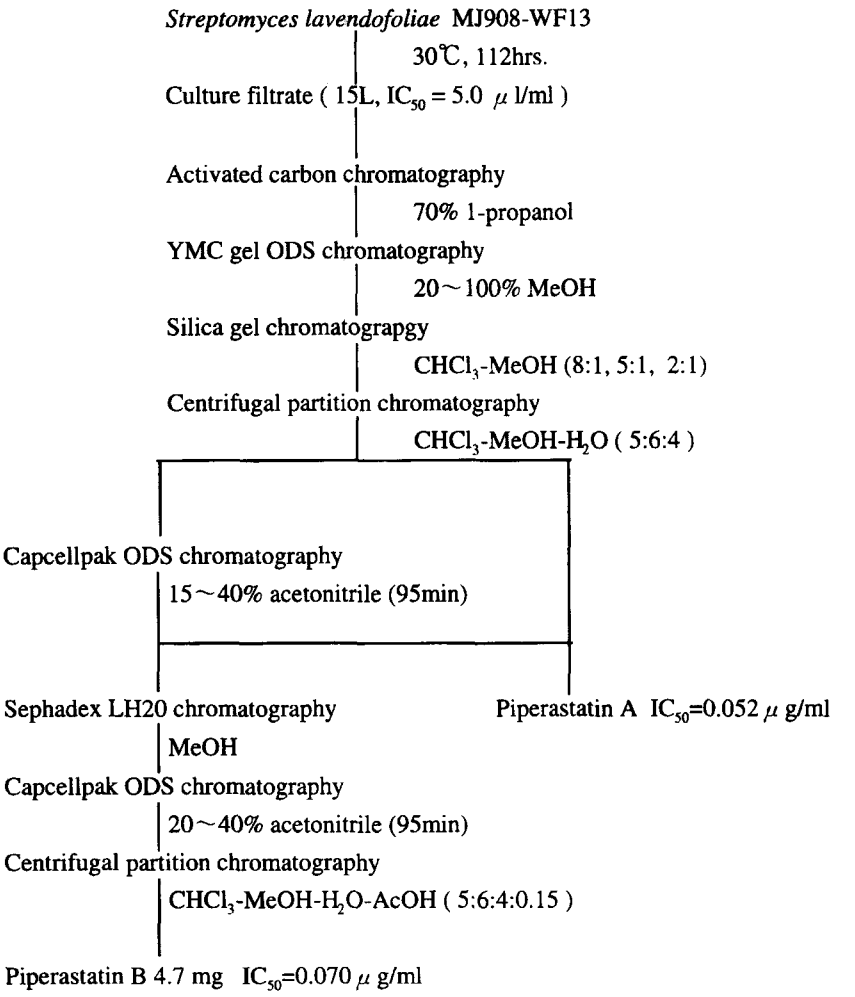


FIGURE 2 Isolation procedure for piperastatin B.

20%–100% MeOH. The active fractions were concentrated under reduced pressure and lyophilized to give a brownish powder (1.18 g). This was dissolved in a solvent mixture of $CHCl_3$-MeOH (8:1) and applied to a column of silica gel (150 ml). The column was washed with $CHCl_3$-MeOH (8:1) and eluted with $CHCl_3$-MeOH (5:1 and then 2:1). The active fractions were concentrated under reduced pressure to give a brownish material (440 mg) containing piperastatins A and B.

The brownish material was subjected to a centrifugal partition chromatography (CPC). The chromatography was performed using a CPC apparatus model NMF (Sanki Engineering Ltd.) under the following conditions: solvent system, $CHCl_3$-MeOH-H_2O (5:6:4); ascending mode; flow rate, 5 ml/min; centrifugation, 700 rpm; temperature, 20°C. The active fractions were concentrated under reduced pressure to give a colourless residue (36.7 mg). The residue was subjected to HPLC. The chromatography was peformed under the following conditions: column, Capcellpak C-18 (20 × 250 mm, Shiseido Co. Ltd.); flow rate, 6.0 ml/min; gradient, 15 ~ 40% aqueous acetonitrile for 95 min; detection, UV at 210 nm. The active fractions were concentrated under reduced pressure and lyophilized to give a white powder (10.4 mg), which was free from piperastatin A. This powder dissolved in MeOH was applied to a column of Sephadex LH20 (350 ml) and eluted with MeOH. The active fractions were evaporated to dryness to give a colourless material (9.1 mg). Final purifications were performed by HPLC (under the above conditions except for the gradient, 20 ~ 40% aqueous acetonitrile for 95 min), followed by the CPC (under the above conditions except for the solvent system ($CHCl_3$-MeOH-H_2O-AcOH, 5:6:4:0.15)) to give pure piperastatin B (4.8 mg).

Analytical Instruments

HPLC was performed by a Gilson's HPLC system, equipped with a Waters 991J photodiode array detector. Amino acid analysis was performed on a Hitachi 850 automatic amino acid analyzer. NMR spectra were recorded on a JEOL JNM-A500 NMR spectrometer and mass spectra were obtained using a JEOL JMS-SX102 spectrometer. The UV spectrum was recorded on a Hitachi U-3210 spectrometer. The IR spectrum was measured on a Hitachi Model 260-10 spectrometer. Optical rotation was determined using a Perkin-Elmer 241 polarimeter. Melting point was measured on a Yanaco melting point apparatus MP500D.

Hydrolysis of Piperastatin B

Piperastatin B (0.05 mg) was hydrolyzed at 110°C for 20 h with 6N HCl (0.15 ml) using a vacuum hydrolysis tube (Pierce, Illinois, USA). After removal of the solvents, the hydrolysate was dissolved in 0.25 M lithium citrate buffer (pH 2.2) and subjected to the amino acid analysis. The amino acids in the hydrolysate were determined as 1 mole of threonine and 2 moles each of leucine and valine.

Preparation of Piperastatin B Methylester

Piperastatin B (4.5 mg) in 0.2 ml MeOH was treated with 0.02 ml of 10% trimethylsilyldiazomethane solution in hexane for 30 min at room temperature.

The reaction mixture were evaporated under reduced pressure and separated by preparative silica gel TLC plate ($CHCl_3$-MeOH, 15:1). The crude product obtained was further purified by HPLC: column, Shodex Asahipak ODP-50 (21.5 × 300 mm, Showa Denko Co. Ltd.); flow rate, 6.0 ml/min; solvent, 37.5% aqueous acetonitrile for 50 min and then 39.2% for another 50 min; detection, UV at 210 nm, to give piperastatin B methylester (2.7 mg). The Rf value was 0.48: silica gel TLC ($CHCl_3$-MeOH, 10:1). The molecular weight was 809 by FAB-MS spectrum.

Enzyme Assays

The assays of various enzymes were performed according to the previous reports,[10] and the conditions of enzyme assays are summarised in Table IV.

The principle of assays for carboxypeptidases and ACE activities is based on the spectrometric determination of *N*-benzoylglycine with cyanuric chloride[12] using a microplate reader model 3550 (BIO-RAD). The reaction mixture (total 0.1 ml) for CP-Y consisted of 25 mM sodium phosphate buffer (pH 6.5), 1 mM Bz-Gly-Phe, 2.0 μg/ml enzyme and water or aqueous solution containing the test compound. After incubation at 37°C for 40 min, 6 μl of 1N sodium hydroxide, 50 μl of 0.36 M sodium phosphate buffer (pH 7.2) and 0.15 ml of 2% (W/V) cyanuric chloride in 2-methoxyethanol were successively added to the mixed solution. CP-Y activity was determined by measuring the absorbance at 405 nm.

The percent inhibition was calculated by the formula $(A - B)/A \times 100$, where A is the value obtained in the enzymatic assay without an inhibitor and B is that with an inhibitor. The IC_{50} value is the concentration of inhibitor at 50% inhibition of enzyme activity.

RESULTS AND DISCUSSION

Purification of Piperastatin B

As shown in Figure 2, a minor component, piperastatin B was isolated by several purification steps and obtained as a white powder (3.8 mg) from the culture filtrate (15 litres). Piperastatin B was separated from the major component, piperastatin A by the first CPC and the first HPLC step. The purity of piperastatin B was confirmed by silica gel TLC ($CHCl_3$-MeOH-H_2O, 65:25:2) and HPLC under following conditions: column, Capcellpak C18 S-5 μm (4.6 × 150 mm, Shiseido Co. Ltd.); flow rate, 1.0 ml/min.; gradient, 20%–50% aqueous acetonitrile for 25 min.; detection, UV at 210 nm.

TABLE I Physico-chemical properties of piperastatin B

Appearance	white powder
Melting point	216 ~ 218°C (dec.)
FAB-MS (positive, m/z)	796 $(M + H)^+$
Molecular weight	795
Molecular formula	$C_{37}H_{65}N_9O_{10}$
HRFAB-MS (positive, m/z)	
Found	796.4917 $(M + H)^+$
Calc for $C_{37}H_{66}N_9O_{10}$	
	796.4933
$[\alpha]_D^{23}$ (*c* 0.4, MeOH)	−36.6°
UV λ max,MeOH nm	end absorption
IR ν max cm^{-1}	3400, 3310, 2950, 2940, 2870, 1650, 1540, 1470,
	1450, 1410, 1390, 1360, 1240, 1160, 920
Rf value on TLC	0.36 ($CHCl_3$-MeOH-H_2O, 65:25:2, Silica gel)
Colour reaction	Mo-H_2SO_4, Greig-Leaback
Solubility	soluble in MeOH, EtOH, DMSO

Physico-chemical Properties of Piperastatin B

The physico-chemical properties of piperastatin B are summarised in Table I. The molecular formula was determined to be $C_{37}H_{65}N_9O_{10}$ by HRFAB-MS, ^{13}C NMR and HSQC. The IR spectrum indicated the presence of NH or OH groups (3300 ~ 3400 cm^{-1}) and peptide bonds (1650 and 1540 cm^{-1}). The UV spectrum showed end absorption in MeOH. Piperastatin B is soluble in MeOH, EtOH, DMSO. It gives positive color reaction with Greig-Leaback and phosphomolybdate-H_2SO_4 (Mo-H_2SO_4).

Structure Determination of Piperastatin B

The molecular formula of piperastatin B was established as $C_{37}H_{65}N_9O_{10}$ by HRFAB-MS. All 37 carbon atoms were detected in the ^{13}C NMR and the HSQC spectrum measured in DMSO-d_6. The DEPT spectra revealed the presence of 30 carbons: 9 methyls, 8 methylenes, 13 methines. The ^1H NMR spectrum showed 7 exchangeable proton signals (Another two exchangeable proton signals were not observed).

The amino acid analysis of piperastatin B gave 1 mole of threonine and 2 moles of leucine and valine, and the presence of these amino acid residues was supported

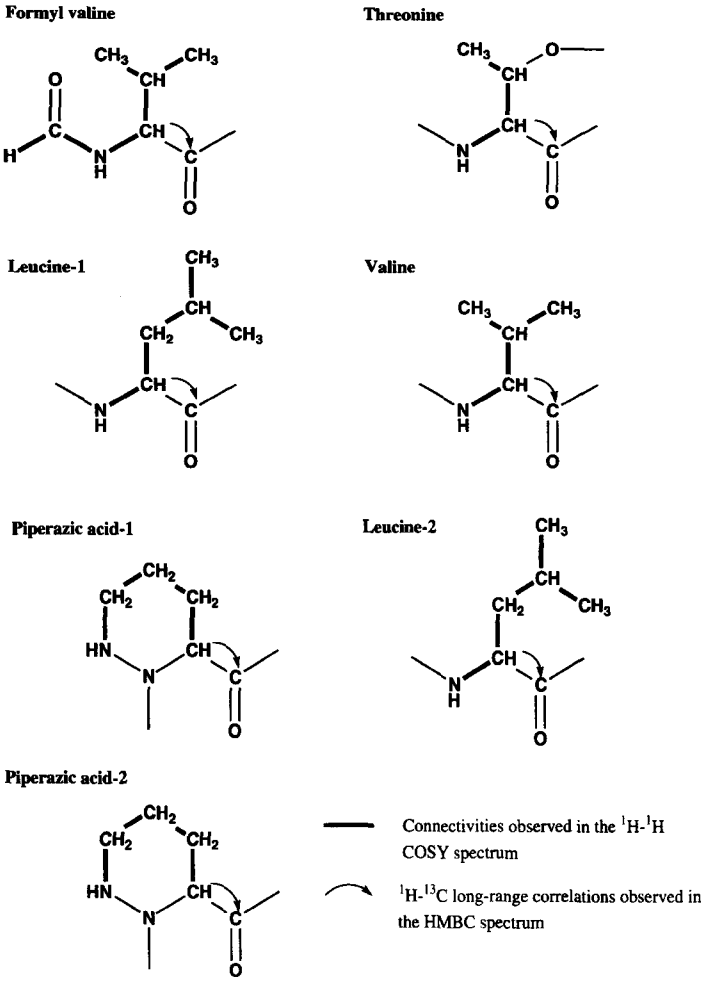


FIGURE 3 Structures of amino acids in piperastatin B elucidated by ^{1}H-^{1}H COSY and HMBC spectra.

by the ^{1}H-^{1}H COSY and HMBC spectra (Figure 3). The ^{1}H-^{1}H COSY spectrum showed that a formyl group masked the amino group of valine-1. The presence of the other components, 2 moles of piperazic acid (hexahydropyridadine-3-carboxylic acid) was also suggested by the ^{1}H-^{1}H COSY and HMBC spectra. From these spectra of piperastatin B methylester, the structure of the same amino acid components were confirmed, as shown in Figure 3. The NMR assignments of piperastatin B and piperastatin B methylester are shown in Table II and Table III respectively.

TABLE II ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) chemical shifts of piperastatin B in DMSO-d_6

	δ_C ppm	δ_H ppm (J in Hz)
Formyl		
CHO	161.0	8.06 d(J=1.0)
Valine-1		
NH		8.26 dd(J=8.9, 1.0)
CO	170.7	
α CH	56.2	4.32 dd(J=8.9, 6.8)
β CH	30.1	2.01 m
γ CH_3	19.1	0.85 d(J=6.8)
γ CH_3	17.7	0.82 d(J=6.8)
Threonine		
NH		7.88 d(J=8.9)
CO	169.9	
α CH	58.2	4.23 dd(8.9, 3.8)
β CH	66.7	3.95 dq(J=6.3, 3.8)
γ CH_3	19.5	1.00 d(J=6.3)
Leucine-1		
NH		7.81 d(J=8.2)
CO	171.5	
α CH	51.1	4.34 m
β CH_2	40.4	1.44 m
γ CH	23.8	1.60 m
δ CH_3	23.1	0.84 d(J=6.7)
δ CH_3	21.2	0.80 d(J=6.7)
Valine-2		
NH		7.60 d(J=9.2)
CO	172.0	
α CH	53.5	5.10 dd(9.2, 6.7)
β CH	30.1	2.01 m
γ CH_3	19.5	0.81 d(J=6.7)
γ CH_3	17.3	0.78 d(J=6.7)
Piperazic acid-1		
CO	170.7	
α CH	49.9	4.93 m
β CH_2	26.5	2.11, 1.65 m
γ CH_2	20.7	1.42 m
δ CH_2	46.6	3.00, 2.53 m
NH		4.92 m

TABLE II *Continued*

	δ_C *ppm*	δ_H *ppm (J in Hz)*
Leucine-2		
NH		7.99 d(J=8.2)
CO	173.2	
α CH	47.1	5.22 m
β CH_2	39.6	1.39 m
γ CH	24.4	1.62 m
δ CH_3	23.2	0.88 d(J=6.6)
δ CH_3	21.3	0.85 d(J=6.6)
Piperazic acid-2		
CO	172.2	
α CH	50.6	4.95 m
β CH_2	25.0	2.11, 1.72 m
γ CH_2	21.3	1.52 m
δ CH_2	46.4	2.94, 2.68 m
NH		4.56 brd(J=12.1)

TABLE III ^1H (500 MHz) and ^{13}C NMR (125 MHz) chemical shifts of piperastatin B methylester in DMSO-d_6

	δ_C *ppm*	δ_H *ppm (J in Hz)*
Formyl		
CHO	161.0	8.06 d(J=1.0)
Valine-1		
NH		8.27 dd(J=9.0, 1.2)
CO	170.5	
α CH	56.2	4.32 dd(J=9.0, 6.2)
β CH	30.1	2.01 m
γ CH_3	19.1	0.86 d(J=6.9)
γ CH_3	17.7	0.82 d(J=6.9)
Threonine		
NH		7.91 d(J=8.8)
CO	169.9	
α CH	58.2	4.23 dd(8.9, 4.0)
β CH	66.7	3.95 m
γ CH_3	19.5	1.00 d(J=6.3)

TABLE III *Continued*

	δ_C *ppm*	δ_H *ppm (J in Hz)*
Leucine-1		
NH		7.82 d(J=8.0)
CO	171.5	
α CH	51.1	4.34 m
β CH_2	40.4	1.46 m
γ CH	23.8	1.59 m
δ CH_3	23.1	0.84 d(J=6.6)
δ CH_3	21.2	0.80 d(J=6.6)
Valine-2		
NH		7.59 d(J=9.3)
CO	172.1	
α CH	53.4	5.10 dd(9.2, 6.1)
β CH	30.2	2.00 m
γ CH_3	19.5	0.81 d(J=6.9)
γ CH_3	17.3	0.78 d(J=6.9)
Piperazic acid-1		
CO	170.5	
α CH	49.9	4.94 m
β CH_2	26.4	2.11, 1.67 m
γ CH_2	20.7	1.43 m
δ CH_2	46.5	3.00, 2.54 m
NH		4.91 m
Leucine-2		
NH		7.99 d(J=8.3)
CO	173.4	
α CH	47.1	5.23 m
β CH_2	39.7	1.41 m
γ CH	24.3	1.63 m
δ CH_3	23.1	0.89 d(J=6.7)
δ CH_3	21.4	0.86 d(J=6.6)
Piperazic acid-2		
CO	170.9	
α CH	50.9	5.05 dd(J=2.3, 5.9)
β CH_2	24.7	2.11, 1.74 m
γ CH_2	21.1	1.42 m
δ CH_2	46.3	2.92, 2.70 m
NH		4.64 dd(J=12.0, 1.7)
OCH_3	52.2	3.69 s

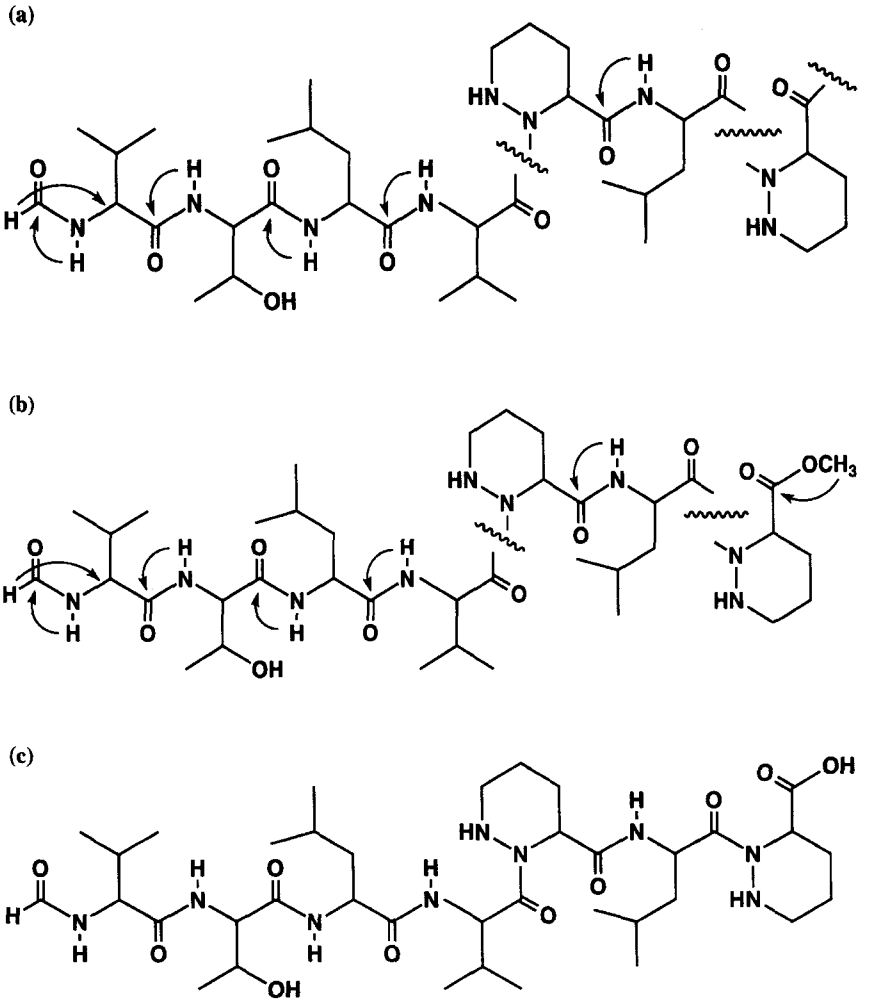


FIGURE 4 Total structure of piperastatin B elucidated by HMBC spectra of piperastatin B and piperastatin B methylester: (a) partial structures of piperastatin B revealed by HMBC experiment, (b) partial structures of piperastatin B methylester revealed by HMBC experiment, (c) total structure of piperastatin B.

TABLE IV Conditions of enzyme assays

Enzyme	*Substrate*	*Other reaction mixture*
CP-Y	1 mM Bz-Gly-Phe	25 mM Na phosphate buffer (pH 6.5)
CP-A	1 mM Bz-Gly-Phe	25 mM Na phosphate buffer (pH 7.5) containing 0.9 M NaCl
CP-B	1 mM Bz-Gly-Lys	25 mM Tris-HCl buffer (pH 8.0)
CP-N	1 mM Bz-Gly-Lys	25 mM Tris-HCl buffer (pH 7.2)
ACE	1.2 mM Bz-Gly-His-Leu	50 mM Tris-HCl buffer (pH 8.0) containing 30 mM NaCl
Leu-AP	0.5 mM Leu-βNA	50 mM Tris-HCl buffer (pH 7.5)
AP-N	0.5 mM Leu-βNA	50 mM Tris-HCl buffer (pH 7.0)
DPP-I	0.4 mM Gly-Arg-βNA	50 mM Citrate buffer (pH 4.0) containing 5 mM NaCl, 7 mM 2-mercaptoethanol
DPP-II	0.4 mM Lys-Ala-βNA	50 mM 3,3-dimethylglutalic acid-NaOH buffer (pH 5.5)
DPP-IV	0.5 mM Gly-Pro-βNA	50 mM Tris-Malate-NaOH buffer (pH 7.0)
α-Chymotrypsin	0.2 mM Suc-L-L-V-Y-MCA	50 mM Tris-HCl buffer (pH 7.5) containing 100 mM NaCl, 1 mM $CaCl_2$
Thrombin	0.2 mM Boc-V-P-R-MCA	50 mM Tris-HCl buffer (pH 7.5) containing 100 mM NaCl
PEP	0.1 mM Z-Gly-Pro-βNA	25 mM Tris-HCl buffer (pH 7.8)
Esterase	0.125 mM PNP acetate	50 mM Na phosphate buffer (pH 7.0) containing 0.03% triton X-100
Lipase	0.125 mM PNP acetate	50 mM Na phosphate buffer (pH 7.0) containing 0.03% triton X-100

The total structure was determined by the HMBC spectra of piperastatin B and piperastatin B methylester. The 5 amide protons in piperastatin B were coupled respectively with 5 amide carbonyl carbons as follows: δ_H 8.26 (valine-1) to δ_C 161.0 (formyl), δ_H 7.88 (threonine) to δ_C 170.7 (valine-1), δ_H 7.81 (leucine-1) to δ_C 169.9 (threonine), δ_H 7.60 (valine-2) to δ_C 171.5 (leucine-1) and δ_H 7.99 (leucine-2) to δ_C 170.7 (piperazic acid-1) and the proton at δ_H 8.06 (formyl) was also coupled to the α carbon at δ_C 56.2 (valine-1). From these connectivities, three partial structures as shown in Figure 4(a) were established. The carboxyterminal amino acid was determined by the HMBC spectrum of piperastatin B methylester in DMSO-d_6. The ^1H-^{13}C long range coupling from the methyl protons at δ_H 3.69 (methylester) to the carbonyl carbon at δ_C 170.9 (piperazic acid-2) revealed that piperazic acid-2 was the carboxyterminal (Figure 4(b)). Thus, the total structure of piperastatin B was determined to be *N*-formyl-Val-Thr-Leu-Val-Pip-Leu-Pip

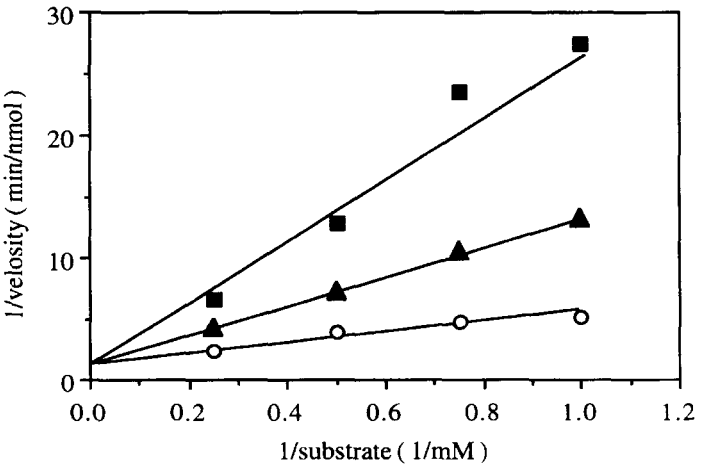


FIGURE 5 Lineweaver-Burk plot of inhibition of CP-Y by piperastatin B: (○) $I = 0$ μM, (▲) $I = 0.125$ μM, (■) $I = 0.252$ μM. I: Inhibitor.

(pip, piperazic acid = hexahydropyridadine-3-carboxylic acid) as shown in Figure 4(c). This compound is an analog of piperastatin A (*N*-formyl-*allo* Ile-Thr-Leu-Val-Pip-Leu-Pip) the major component from the same microorganisms.[11] The absolute configuration of the amino acid residues remains to be elucidated.

Enzymatic Inhibitory Activities of Piperastatin B

The inhibitory activity (IC_{50}) of piperastatin B against CP-Y was determined to be 70 ng/ml (88 nM; MW, 795). The Lineweaver-Burk plot of piperastatin B against CP-Y exhibited competitive inhibition as shown in Figure 5. K_i value was determined to be 55 nM (mean value from duplicate experiments) by Dixon plots of CP-Y inhibition by piperastatin B. These data suggested that piperastatin B showed a potent inhibitory activity against CP-Y as piperastatin A (IC_{50}, 64 nM; K_i, 52 nM).[10] Piperastatin B has low toxicity, since there were no deaths after intraperitoneal injection of mice with 100 mg/kg of piperastatin B (data not shown).

Examination of the inhibitory characteristics of piperastatin B against many kinds of enzymes (see Table IV); metallo carboxypeptidases (CP-A, B and N), dicarboxypeptidase (ACE), aminopeptidases (AP-M and Leu-AP), dipeptidyl-aminopeptidases (DPP-I, II and IV), cystein peptidase (cathepsin B), serine endopeptidases (chymotrypsin, thrombin and PEP), lipase and esterase, showed that a piperastatin B had a high selectivity against CP-Y except for inhibiting

TABLE V Inhibitory activities of piperastatin B against various enzymes

Enzymes	*Origin*	IC_{50} ($\mu g/ml$)
CP-Y	yeast	0.070
CP-A	bovine pancreas	>100
CP-B	porcine pancreas	>100
CP-N	human plasma	>100
ACE	bovine lung	40
Leu-AP	hog kidney	>100
AP-N	hog kidney	>100
DPP-I	rat spleen	>100
DPP-II	rat spleen	>100
DPP-IV	rat kidney	>100
Chymotrypsin	bovine pancreas	>100
Thrombin	human plasma	>100
PEP	hog kidney	>100
Cathepsin B	bovine spleen	>100
Lipase	hog pancreas	>100
Esterase	hog liver	>100

ACE weakly, as shown in Table V. These data revealed that piperastatin B as well as the major component, piperastatin A had the characteristics of a potent and selective inhibitor of CP-Y, as described in a previous paper.[10]

In the previous work, we discovered belactins A and B[13,14] and piperastatin A[10,11] as novel selective serine carboxypeptidase inhibitors from Actinomycete. In this work, we isolated piperastatin B, a new serine carboxypeptidase inhibitor as a minor component from *Streptomyces lavendofoliae* MJ908-WF13 and determined its structure as *N*-formyl-Val-Thr-Leu-Val-Pip-Leu-Pip (pip, piperazic acid = hexahydropyridadine-3-carboxylic acid). Piperastatin B has high selectivity against CP-Y and is considered to be a useful tool for understanding the roles of serine carboxypeptidases such as CP-Y, platelet deamidase or CP-Y like kininase.

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