THE STRUCTURE AND ACTIVITY OF LEUPEPTINS AND RELATED ANALOGS

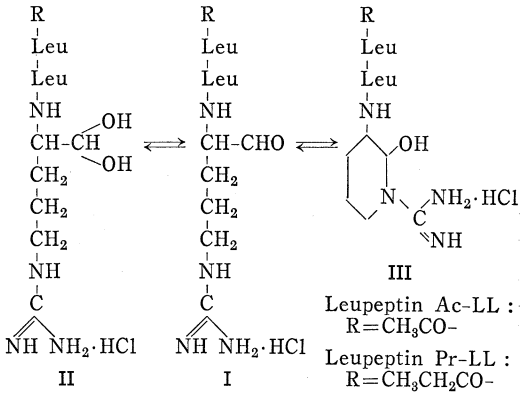
Sir:

Leupeptins produced by various species of *Actinomycetes*, strongly inhibit proteases such as plasmin, trypsin and papain[1,2,3]. We previously reported[4] the structures of two major components, acetyl-L-leucyl-L-leucyl-DL-argininal (leupeptin Ac-LL) and propionyl-L-leucyl-L-leucyl-DL-argininal (leupeptin Pr-LL), confirmed by chemical syntheses. We have also suggested[3] that various leupeptin analogs which contain L-isoleucine or L-valine instead of L-leucine might show interesting biological activity. In this communication, the syhtheses and biological activities of leupeptin analogs and the evidence for the equilibrium structures of leupeptins are reported.

The analogs of leupeptins, shown in Table 1, were synthesized by reduction of the corresponding acyl peptide esters with lithium borohydride followed by oxidation with the PFITZNER-MOFFATT reagent[5] as described previously[4]. N^{α}-Acetylargininal was synthesized from N^{α}-acetylargininol derived from L-arginine. The activity was evaluated by measuring the inhibition of plasmin fibrinogenolysis, papain caseinolysis and thrombokinase according to the method of AOYAGI *et al.*[2]

As reported previously[2], the derivatives of leupeptins Ac-LL and Pr-LL with terminal carboxylic acid, alcohol or di-*n*-butyl acetal groups instead of aldehyde exhibited no effect on fibrinogenolysis by plasmin. As shown in Table 1, acetyl-L-valyl-L-leucyl-DL-argininal and acetyl-L-isoleucyl-L-leucyl-DL-argininal inhibit proteolysis by plasmin more strongly than leupeptins Ac-LL and Pr-LL. These results indicate that the aldehyde group plays an essential role for activity and compounds with leucyl-argininal sequence are strongly active.

A signal at δ 9.9 ppm in the NMR spectrum of leupeptin Ac-LL hydrochloride or Pr-LL hydrochloride in D_2O, can be reasonably assigned to the aldehyde proton (very weak), and two doublet signals at δ 5.5 (J=4.5 Hz) and δ 5.9 (J=3 Hz) correspond to two kinds of protons, although their integral is less than that for one proton (about half). As reported earlier[1,3], leupeptins have reducing property (red tetrazolium) and also show a positive SAKAGUCHI reaction (characteristic of monosubstituted guanidine). Leupeptin Ac-LL or Pr-LL was separated into two spots (Rf 0.35~0.45 and 0.30~0.35 or 0.45~0.50 and 0.35~0.45, respectively) on thin-layer chromatography on Silica Gel G (Merck) with butanol - butyl acetate - acetic acid - water (4 : 2 : 1 : 1, v/v) as a developing solvent. These data can be explained by assuming an equilibrium mixture of aldehyde (I), the hydrate form (II) and carbinolamine forms or hydroxypiperidine ring (III) in aqueous solution as shown below.



Leupeptin Ac-LL : R=CH_3CO-

Leupeptin Pr-LL : R=CH_3CH_2CO-

The evidence for such equilibrium has also been confirmed by NMR data on synthetic N^{α}-acetylargininal and acetyl-L-leucyl-DL-argininal, as shown in Table 2. A signal around δ 5.5 with J=4.5 Hz of leupeptins and synthetic analogs can be assigned to a hydrated aldehyde proton on the basis of NMR spectral behavior of *n*-propanal in D_2O*. The carbinolamine formation of a six-membered ring from δ-amino aldehyde compounds is seen with 5-aminosugars[6,7,8] and β-guanidinopropionaldehyde[9]. From the analysis of the NMR spectrum of nojirimycin sulfate (D-glucopiperidinose)[7,8] in D_2O

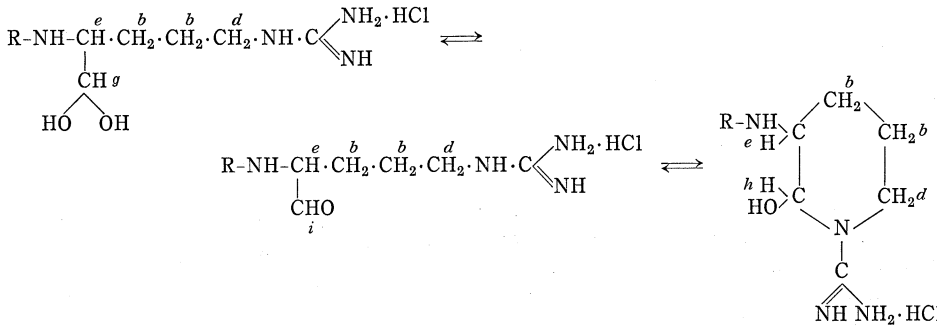
* The NMR spectrum of *n*-propanal in D_2O was measured under the same condition as leupeptins, showing the free aldehyde proton and the hydrated aldehyde proton at δ 10.1 (J=15 Hz, 0.5 H) and 5.4 (J=5 Hz, 0.5 H), respectively.

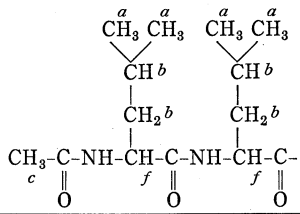
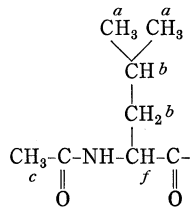
Table 1. Biological activity of leupeptins and some analogs

Compound (Hydrochloride)	m. p. (°C)	$[\alpha]_D$	ID_{50} (mcg/ml)		
			Fibrinogenolysis by plasmin	Caseinolysis by papain	Thrombokinase
N^{α}-Ac-Argal		−12.5° (*c* 2, H_2O, 22°C)	>333		
Ac-Leu-Argal	75~85	−32° (*c* 1, CH_3OH, 29°C)	8	0.3	38
Pr-Leu-Argal	105~130	−30° (*c* 1, H_2O, 19°C)	8		
Ac-Ileu-Argal	85~110	−58° (*c* 2, H_2O, 30°C)	33		
Ac-Leu-Leu-Argal (Leupeptin Ac-LL)	65~100	−42° (*c* 1, CH_3OH, 28°C)	9	0.8	14
Pr-Leu-Leu-Argal (Leupeptin Pr-LL)	79~90	−46° (*c* 3, CH_3OH, 21°C)	8	0.5	14
Ac-Ileu-Leu-Argal	125~135	−60° (*c* 2, H_2O, 22°C)	2	2.0	11
Ac-Val-Leu-Argal	85~100	−53° (*c* 1, CH_3OH, 29°C)	2	1.7	9
Ac-Ileu-Ileu-Argal	145~150	−47° (*c* 1, H_2O, 22°C)	35	6.5	
Ac-Ileu-Val-Argal	145~150	−27° (*c* 1, H_2O, 22°C)	314	15.5	

Abbreviations: Ac: acetyl, Pr: propionyl, Leu: L-leucyl, Ileu: L-isoleucyl, Val: L-valyl, Argal: argininal

Table 2. NMR spectra of leupeptin Ac-LL, acetyl-L-leucyl-DL-argininal and N^{α}-acetylargininal in D_2O (100 MHz, tetramethylsilane as external standard)



	Leupeptin Ac-LL hydrochloride				Acetyl-L-leucyl-DL-argininal hydrochloride				N^{α}-Acetylargininal hydrochloride			
R												
	δ (ppm)	Type	H	J (Hz)	δ (ppm)	Type	H	J (Hz)	δ (ppm)	Type	H	J (Hz)
a	1.4	d. d	12		1.4	d. d	6					
b	1.9~2.4	broad	10		1.9~2.4	broad	7		2.0~2.4	broad	4	
c	2.52	s	3		2.50	s	3		2.58	s	3	
d	3.6~4.1	broad	2		3.6~4.1	broad	2		3.5~4.1	broad	2	
e	4.2~4.5	broad	1		4.2~4.6	broad	1		4.2~4.6	broad	1	
f	4.7~5.0	broad	2		4.75	t	1					
g	5.5	d	0.5	4.5	5.4	d	0.5	4.5	5.5	d	0.5	4.5
h	5.9	d	0.5	3	5.9	d	0.5	3	6.0	d	0.5	3
i	9.9		trace		9.9		trace		9.9		trace	

(sodium 2,2-dimethyl-1,2-silapentane-5-sulfonate as internal standard), INOUYE reported that signals at τ 4.72 (J=3.2 Hz) and τ 5.36 (J=7.0 Hz) could be assigned to α- and β-anomeric protons, respectively. Therfore, a signal around δ 6.0 with J=3 Hz can be assigned to the carbinolamine proton of the hydroxypiperidine ring. The NMR data suggest the presence of **II** and **III** at a higher concentration than **I**.

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