HYDROXYPEPSTATIN, A NEW PEPSTATIN PRODUCED BY STREPTOMYCES

Sir:

In previous papers, we reported isolation and structure determination of pepstatin[1,2], isovaleryl-L-valyl-L-valyl-(3S, 4S)-4-amino-3-hydroxy-6-methylheptanoyl-L-alanyl-(3S, 4S)-4-amino-3-hydroxy-6-methylheptanoic acid, and its analogs[3,4] in which 2- to 8-carbon fatty acids replace isovaleric acid as well as the pepstanones[3], compounds thought to be derived from the pepstatins by oxidative decarboxylation. Pepstatin was designated pepstatin A. Various pepstatins and pepstanones showed a similar activity against pepsin[5,6] and in addition inhibited cathepsin D and renin[7,8]. In this communication, we report isolation of hydroxypepstatin A in which serine replaces alanine.

Hydroxypepstatin A was produced together with other pepstatins by shaking culture or tank fermentation of *Streptomyces testaceus* in media containing various kinds of carbon and nitrogen sources. A typical medium used for the production contained 5% glucose, 4.5% peptone, 0.15% K_2HPO_4, 0.3% NaCl and 0.1% $MgSO_4 \cdot 7H_2O$. Production of hydroxypepstatin A reached the maximum after 120 hours in a tank fermentation. The culture filtrate (120 liters) was extracted with *n*-butanol (60 liters) at pH 2.0. The *n*-butanol extract was washed with 0.1N NaOH (15 liters), adjusted to pH 5.0 with dil. H_2SO_4, and concentrated under reduced pressure. The concentrated solution (1.5 liters) yielded a yellowish powder (175g), after being held at 4°C for 20 hours. This crude powder (20g) containing the pepstatins and hydroxypepstatin A was dissolved in 90% methanol (2 liters), and passed through a column of Dowex 1×2 (acetate form, 0.5 liters). After the column was washed with 90% methanol (1.0 liter), pepstatins and hydroxypepstatin A were eluted with 90% methanol containing 0.12N triethylamine hydrochloride. Hydroxypepstatin A appeared in the fractions obtained following elution of the pepstatins. The fraction containing hydroxypepstatin A was dried under reduced pressure at below 40°C and suspended in a mixture of *n*-butanol (300 ml) and water (200 ml). After the pH was adjusted to 2.5 with dil. H_2SO_4, the *n*-butanol layer was separated and concentrated under reduced pressure at below 40°C yielding a white powder (3g). The white powder (0.6g) was dissolved in 70% methanol (30 ml), and passed through a column of Amberlite XAD-2 (50~100 mesh, 0.6 liters). Hydroxypepstatin A was eluted with 70% methanol, thereby completely separating it from contaminating pepstatins present in the later fractions. After evaporation of the fraction containing hydroxypepstatin A, the latter was crystallized from *n*-butanol (125 mg).

Hydroxypepstatin A was obtained as colorless crystals, m.p. 232.5~233.5°C (dec.). Anal. calcd. for $C_{34}H_{63}N_5O_{10} \cdot H_2O$: C56.72, H9.10, N9.72; found C56 69, H9.23, N9.66. The molecular weight was confirmed by mass spectrometry: TMS-hydroxypepstatin A methyl ester M^+, *m/e* 931. The UV spectrum of hydroxypepstatin A showed only end absorption. The infrared spectrum is shown in Fig. 1. On silica gel thin-layer chromatography developed with chloroform-methanol-acetic acid (86:12:2), the Rf value of hydroxypepstatin A detected by RYDON-SMITH reagent was 0.20, while that of pepstatin A was 0.35.

Hydroxypepstatin A was hydrolyzed in 6N HCl at 105°C for 16 hours. The amino acid

Fig. 1. Infrared absorption spectrum of hydroxy pepstatin A (KBr)

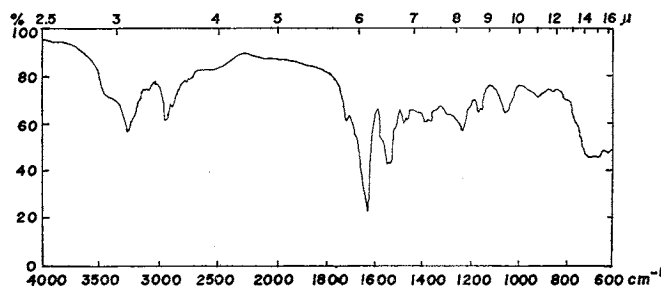


Fig. 2. The mass spectrum of TMS-hydroxypepstatin A-OMe

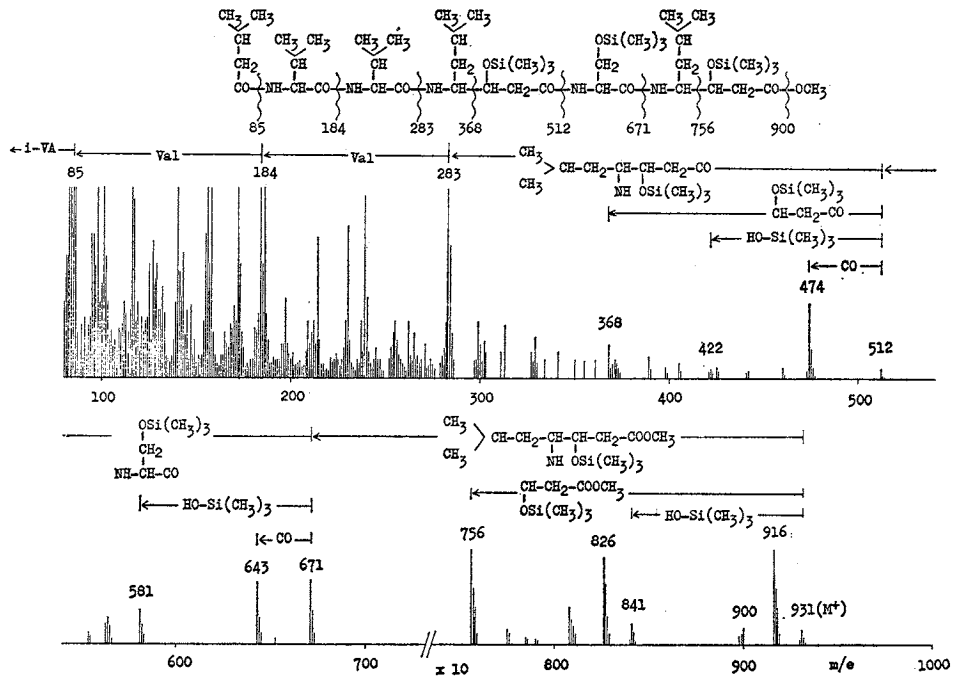


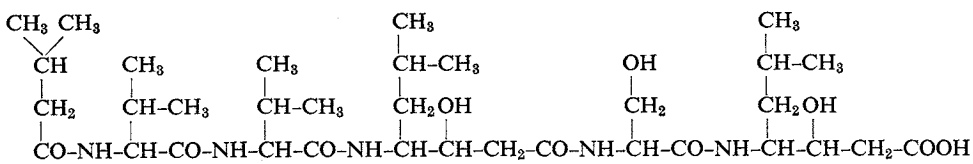
Table 1. The inhibitory activity of hydroxy pepstatins against pepsin, cathepsin D and renin

	Molecular weight	Inhibitory activity (ID_{50})		
		Pepsin ($\times 10^{-8}$ M)	Cathepsin D ($\times 10^{-9}$ M)	Renin ($\times 10^{-6}$ M)
Pepstatin A	685	1.4	8.8	6.6
Hydroxypepstatin	701	1.7	11.4	20.2

The inhibitory activity against pepsin, cathepsin D and renin were carried out as described in previous papers[7].

analysis indicated the presence of one mole of serine, two moles of valine, and two moles of 4-amino-3-hydroxy-6-methylheptanoic acid. The presence of isovaleric acid in the hydrolyzate was confirmed by gas chromatography of its methyl ester.

The mass spectrum of O-trimethylsilyl hydroxypepstatin A methyl ester is shown in Fig. 2. The fragmentation patterns indicated clearly the following sequence:



The activity of hydroxypepstatin A in inhibiting pepsin, cathepsin D and renin is shown in Table 1. Hydroxypepstatin A was equally inhibitory to pepsin and cathepsin D as pepstatin A, but slightly less inhibitory to renin. Gas chromatography of a hydrolysate of a crude-preparation of hydroxypepstatin A obtained without recrystallization suggested the presence of hydroxypepstatin analogs differing in the fatty acid moiety.

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