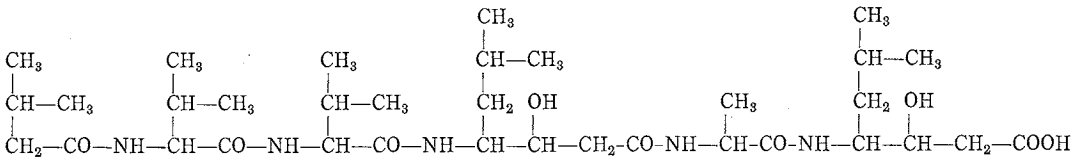
THE CHEMICAL SYNTHESIS OF PEPSTATIN A

Sir:

Pepstatin A is a pepsin inhibitor isolated from cultured broths of *Streptomyces testaceus* HAMADA *et* OKAMI and *Streptomyces argenteolus* var. *toyonakensis*[1,2]. It is the N-*iso* valeryl derivative of a pentapeptide composed of one mole of L-alanine, two moles of L-valine and two moles of 4-amino-3-hydroxy-6-methylheptanoic acid (abbreviated as AHMHA) which is a new naturally-occurring amino acid. The amino acid sequence was determined by mass spectrometry as follows[3]. In this communication, we report the chemical synthesis of pepstatin A.



In determining synthetic strategy, the following was considered. The protection of the amino of AHMHA with carbobenzoxyl group was undesirable because of easy formation of the 2-oxazolidone derivative by participation of neighbouring hydroxyl group. The carboxylic acid-blocking group for AHMHA should be removed by catalytic hydrogenolysis because AHMHA might be dehydrated in the process of saponification. So we chose to use AHMHA with the free amino, with the carboxyl blocked by the *p*-nitrobenzyl ester, which was more stable than the benzyl ester when the carbobenzoxyl group was removed by treatment with hydrogen bromide in acetic acid[4].

The scheme of chemical synthesis of pepstatin A is shown in Fig. 1.

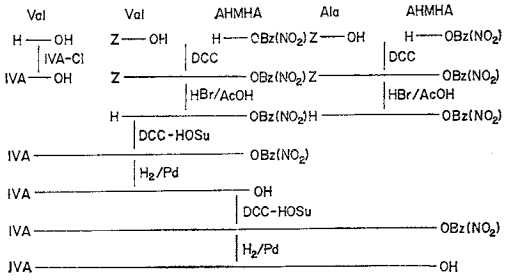
The *p*-nitrobenzyl ester of AHMHA was prepared by azeotropic distillation procedure. A mixture of 1 eq. of AHMHA, 1.4 eq. of *p*-nitrobenzylalcohol and 1.5 eq. of *p*-toluene sulfonic acid in carbon tetrachloride was refluxed for two days with DEAN-STARK adaptor. After removal of the solvent, the formed ester was crystallized from methanol and ether (76 % yield).

Carbobenzoxyvaline and AHMHA *p*-nitrobenzyl ester were coupled by the DCC method. Carbobenzoxyvaline in dichloromethane was cooled to 0°C and an equivalent of dicyclohexylcarbodiimide was added. Thirty minutes after addition, *p*-nitrobenzyl AHMHA *p*-toluene sulfonate and triethylamine were added. The reaction mixture was stirred for 15 hours at 0°C. After removal of dicyclohexylurea by filtration, the filtrate was evaporated under reduced pressure. The product was purified by silica gel column chromatography using benzene-ethyl acetate (2:1) (63 % yield). Carbobenzoxyalanyl-AHMHA *p*-nitrobenzyl ester was prepared by the same method (60 % yield).

Iso-valeryl-valine was prepared with *iso*-valeryl chloride and valine by SCHOTTEN-BAUMANN reaction.

Hydrogen bromide in acetic acid was used to remove the carbobenzoxyl group. The *p*-nitrobenzyl ester of carbobenzoxyvalyl-AHMHA was dissolved in acetic acid containing 25 % HBr at 0°C and 40 minutes later dry ether was added to precipitate valyl-AHMHA *p*-nitrobenzyl ester hydro-

Fig. 1. The scheme of the synthesis of pepstatin A



Abbreviations: IVA, *iso*-valeric acid; Val, L-valine; AHMHA, 4-amino-3-hydroxy-6-methylheptanoic acid; Ala, L-alanine; Z, carbobenzoxy; Bz(NO_2), *p*-nitrobenzyl; DCC, dicyclohexylcarbodiimide; HOSu, N-hydroxy succinimide.

bromide. It was washed with ether (90 % yield). Alanyl-AHMHA *p*-nitrobenzyl ester hydrobromide was prepared the same way (95 % yield).

Valyl-AHMHA *p*-nitrobenzyl ester hydrobromide was dissolved in dimethylformamide, and 1.0 eq. of triethylamine, 1.2 eq. of *iso*-valeryl-valine, 2.0 eq. of N-hydroxy succinimide and 1.1 eq. of dicyclohexylcarbodiimide were added at −16°C. The reaction mixture was stirred for 2 days at 0°C and 1 day at room temperature. After removal of dicyclohexylurea by filtration, the filtrate was dried. The residue was washed with a small amount of methanol giving colorless *iso*-valeryl-valyl-valyl-AHMHA *p*-nitrobenzyl ester (47 % yield). The *p*-nitrobenzyl ester was readily removed by catalytic hydrogenolysis with 10 % palladium-on-charcoal in dimethylformamide solution containing a small amount of acetic acid. After removal of catalyst by filtration, the solution was dried up, and the formed *p*-toluidine was removed by washing of ether.

The final coupling was done between *iso*-valeryl-valyl-valyl-AHMHA and alanyl-AHMHA *p*-nitrobenzyl ester hydrobromide under the same procedure just described above. The product was purified by silica gel column chromatography with chloroform, methanol and acetic acid (95.5 : 3 : 1.5) (70 % yield). After removal of *p*-nitrobenzyl ester by hydrogenolysis, the synthesized pepstatin was crystallized from methanol.

The elemental analysis agreed with the calculated value. Found: C 59.11, H 9.22, N 10.16. Calcd. for $C_{34}H_{63}N_5O_9$: C 59.53, H 9.25, N 10.21. It decomposed at 226～229°C (natural: 228～229°C[3]). The optical rotation of synthetic pepstatin A (*c* 0.1, methanol) was $[\alpha]_{365}^{26}$ −295°, $[\alpha]_{405}^{26}$ −220°, $[\alpha]_{436}^{26}$ −177°, and $[\alpha]_{546}^{26}$ −100°, while that of natural material was $[\alpha]_{365}^{26}$ −298°, $[\alpha]_{405}^{26}$ −222°, $[\alpha]_{436}^{26}$ −183°, and $[\alpha]_{546}^{26}$ −102°. The IR spectrum was the same as the natural product and the inhibitory activity against pepsin was also the same, indicating a high degree of optical purity for the synthetic material.

Acknowledgement

We are grateful to Dr. RINZO NISHIZAWA, Research Laboratory, Nippon Kayaku Co., Ltd., for discussions.

HAZIME MORISHIMA
TOMOHISA TAKITA
HAMAO UMEZAWA

Institute of Microbial Chemistry,
Kamiosaki, Shinagawa-ku,
Tokyo, Japan

(Received June 14, 1972)

References

1) UMEZAWA, H.; T. AOYAGI, H. MORISHIMA, M. MATSUZAKI, M. HAMADA & T. TAKEUCHI: Pepstatin, a new pepsin inhibitor produced by actinomycetes. J. Antibiotics 23: 259～262, 1970
2) MIYANO, T.; M. TOMIYASU, H. IIZUKA, S. TOMISAKA, T. TAKITA, T. AOYAGI & H. UMEZAWA: New pepstatins, pepstatins B and C, and pepstanone A, produced by streptomyces. J. Antibiotics 25: 489～500, 1972
3) MORISHIMA, H.; T. TAKITA, T. AOYAGI, T. TAKEUCHI & H. UMEZAWA: The structure of pepstatin. J. Antibiotics 23: 263～265, 1970
4) SCHWARZ, H. & K. ARAKAWA: The use of *p*-nitrobenzyl esters in peptide synthesis. J. Am. Chem. Soc. 81: 5691～5695, 1959