# SYNTHESIS OF 4-AMINO-3-HYDROXY-6-METHYLHEPTANOIC ACID, AN AMINE COMPONENT OF PEPSTATIN

Sir:

In this communication we report the synthesis of 4-amino-3-hydroxy-6-methylheptanoic acid (AHMHA), an amine moiety of the pepstatins. Various pepstatins, pepsin inhibitors produced by *Streptomyces*, are different from one another in their N-acyl moieties.[1,2] Pepstatin A, *iso*-varelyl-L-valyl-L-valyl-AHMHA-L-alanyl-AHMHA, was chemically synthesized from its components.[3] Combined with the chemical synthesis reported in the previous paper, the synthesis of AHMHA means the total synthesis of pepstatin A has been accomplished.

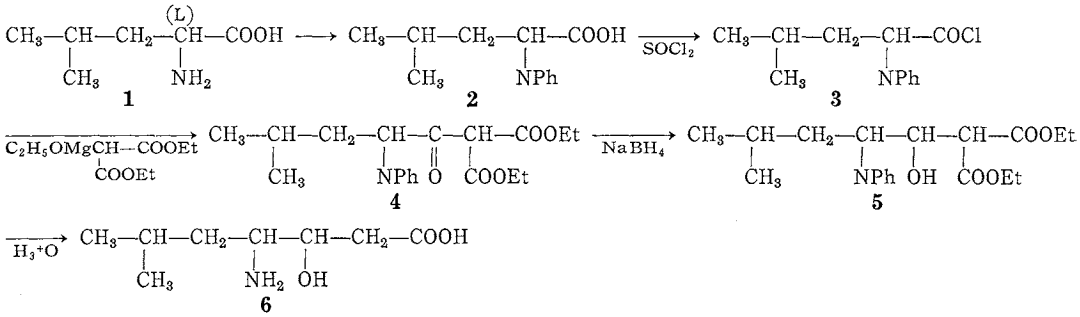
4-Amino-6-methyl-*trans*-2-heptenoic acid, the dehydrated product of AHMHA, which was produced during acid hydrolysis of pepstatin, was acetylated by acetic anhydride in methanol. Oxidation of the acetylated product with LEMIEUX's reagent[4] ($KMnO_4$-$NaIO_4$) followed by acid hydrolysis gave L-leucine in 54 % yield. From the viewpoint of peptide biosynthesis[5], the stereochemistry (S-configuration) of $C_4$ of AHMHA thus determined suggested a possible biogenetic pathway from L-leucine and acetate to AHMHA. Simulating the biogenesis, we designed the chemical synthesis as shown by the scheme in Fig. 1.

The amino group of L-leucine was protected by a phthalyl group.[6] N-Phthalylleucine was dissolved in benzene and reacted with thionyl chloride at room temperature for 24 hours. It was further refluxed for one hour. N-Phthalylleucyl chloride (**3**) was obtained in almost quantitative yield. The benzene solution of **3** was added dropwise to a refluxing ether solution of the magnesium derivative of diethylmalonate. Reflux was continued further for 5 hours after the addition. The reaction mixture was washed with cold dilute sulfuric acid and then evaporated. Compound **4** was obtained in 82 % yield. The NMR spectrum showed the presence of small amounts of contaminants (about 7 mole per cent of diethylmalonate and phthalylleucine). The crude **4** was used for further studies without purification.

Monodecarboxylation of **4** was tried by reflux in xylene solution containing *p*-toluene sulfonic acid. In this treatment, the optically active carbon was racemized. Therefore, the keto group of **4** was first reduced with $NaBH_4$ in benzene solution at room temperature. The IR and NMR spectra of the product (**5**), which was obtained in very good yield, indicated that the other carbonyl groups remained unchanged. Reflux of **5** in 4 N HCl gave the decarboxylated product (**6**) without the protective group. As expected, **6** was a mixture of AHMHA and its diastereoisomer. The NMR spectrum indicated that the yields of both products were almost the same.

AHMHA was separated from the diastereoisomer by column chromatography with a strongly acidic ion-exchange resin (Dowex 50) and a 0.1 M pyridine-acetate buffer of pH 5.0. The diastereoisomer was eluted faster than AHMHA. The synthetic AHMHA was optically pure: $[\alpha]_{365}^{21}$ (*c* 1, $H_2O$) −49°, $[\alpha]_{405}$ −40, $[\alpha]_{436}$ −33°, $[\alpha]_{541}$ −20°, natural; $[\alpha]_{365}^{21}$ (*c* 1, $H_2O$) −49°, $[\alpha]_{405}$ −40°, $[\alpha]_{436}$ −34°, $[\alpha]_{541}$ −20°.

Fig. 1. Synthetic scheme of 4-amino-3-hydroxy-6-methylheptanoic acid.
Abbreviation: Ph, phthalyl



The diastereoisomer showed the same behavior as AHMHA in cellulose thin-layer chromatography and in paper electrophoresis. The NMR spectrum was slightly different from AHMHA: the diastereoisomer, $C_3$-H $\delta$ 4.72, $C_4$-H 3.90, $J_{3-4}$ 3.3 Hz; AHMHA, $C_3$-H $\delta$ 4.46, $C_4$-H $\delta$ 3.47, $J_{3-4}$ 5.5 Hz, in $D_2O$, external TMS reference.

The stereochemistry of AHMHA is now being studied by X-ray crystallography.

HAJIME MORISHIMA
TOMOHISA TAKITA
HAMAO UMEZAWA

Institute of Microbial Chemistry,
14-23, Kamiosaki 3-chome,
Shinagawa-ku, Tokyo, Japan

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