CHEMISTRY OF BLEOMYCIN. XIV*

BIOGENETIC-LIKE SYNTHESIS OF (2S, 3S, 4R)-4-AMINO-3-HYDROXY-2-METHYL-*n*-VALERIC ACID, AN AMINE COMPONENT OF BLEOMYCIN

Sir:

The stereochemistry of 4-amino-3-hydroxy-2-methyl-*n*-valeric acid (**I**), an acid hydrolysis product of bleomycin, was established as (2S, 3S, 4R) by X-ray crystallographic analysis and chemical studies[1]. Biogenetically, **I** seems to be derived from L-alanine and propionate, which are transformed to D-alanine and methylmalonate and then coupled with each other.

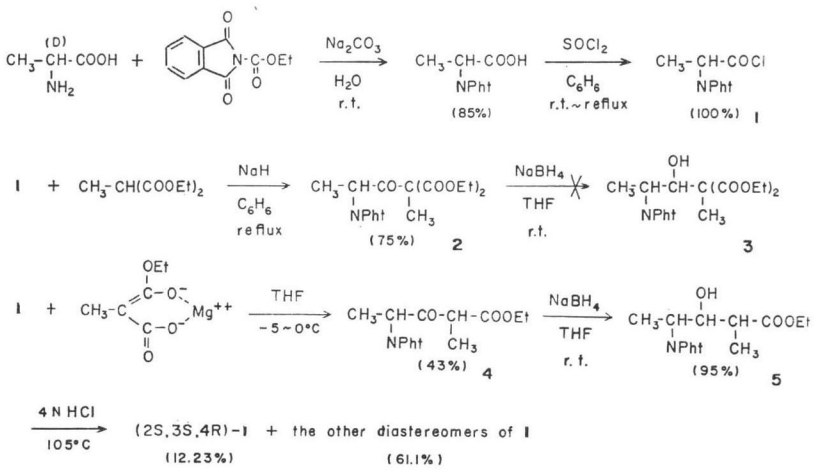
Recently, we reported the synthesis of a biogenetically similar compound: (3S, 4S)-4-amino-3-hydroxy-6-methylheptanoic acid (AHMHA), an amine component of pepstatin, from L-leucine and malonate[2]. Later, biosynthetic studies of pepstatin proved that AHMHA is biosynthesized from L-leucine and malonate by stepwise incorporation during the peptide chain elongation of pepstatin[3].

We planned a biogenetic-like synthesis of (2S, 3S, 4R)-**I** starting from D-alanine and methylmalonate, and first followed the same synthetic route as that of AHMHA[2]. That is: N-phthaloyl-D-alanyl chloride was coupled with diethyl methylmalonate in the presence of sodium hydride. The product (**2** in Fig. 1) was obtained in 75% yield. Compound **2** was treated with sodium borohydride in benzene at room temperature to reduce the keto group, but the reaction did not proceed at all. In the case of synthesis of AHMHA, the yield in this reduction process was almost quantitative under the same conditions. In tetrahydrofuran solution, **2** reacted with $NaBH_4$ at room temperature, but the expected product (**3**) was not obtained and one of the products was 2-phthalimino-*n*-propanol. It is conceivable that the presence of three substitution groups in the vicinity of the keto group blocked the expected reaction.

Then, N-phthaloyl-D-alanyl chloride was reacted with the half ester of methylmalonic acid in the presence of two equivalents of isopropyl magnesium bromide in tetrahydrofuran at $-5°\sim0°C$ for 4 hours. After chromatographic purification, the coupled product (**4**), a diastereoisomeric mixture, was obtained in 43% yield, M^+ 303. This coupling process is accompanied with decarboxylation like a biogenetic coupling.

The selective reduction of the keto group of **4** was accomplished with $NaBH_4$ in tetrahydrofuran at room temperature in 95% yield. The reduced product (**5**) was expected to be a mixture of four diasteromers Separation of the diastereomers was tried after

Fig. 1. Synthetic scheme of 4-amino-3-hydroxy-2-methyl-*n*-valeric acid (I)
Abbreviation: Pht, phthaloyl; r.t., room temperature; THF, tetrahydrofuran



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removal of the protecting groups by acid hydrolysis.

Liquid chromatography using Aminex A 7, a sulfonic acid resin, was used to study the composition of the four diastereomers in the product and the best conditions to separate (2S, 3S, 4R)-**I** from the other three diastereomers. Using a column of 2.2 mm×600 mm, with 0.1 M pyridine-acetate buffer of pH 5.00 as the developing solvent at 90°C, the four isomers were well separated as monitored with an RI-detector. Composition of the isomers was calculated from the integrated areas of the peaks assuming each component shows the same concentration-RI relationship. Composition and retention time at flow rate 0.5 ml/min. (176 kg/cm²) were as follows: peak 1 [(2S, 3S, 4R)-**I**] 20%, 30 minutes; peak 2 27%, 33 minutes; peak 3 38%, 37 minutes; peak 4 15%, 39 minutes.

The best conditions to separate (2S, 3S, 4R)-**I** from the other three diastereomers were 0.2 M pyridine-acetate buffer of pH 3.75 at 31°C. The retention time of (2S, 3S, 4R)-**I** at flow rate 0.3 ml/min. (239 kg/cm²) was 28 minutes, and the other three diastereomers appeared as an unseparated single peak at 42 minutes.

With this information, preparative separation was efficiently achieved by column chromatography on Dowex 50 W, a sulfonic acid resin, using 0.2 M pyridine-acetate buffer at pH 3.75 at room temperature. The yield of (2S, 3S, 4R)-**I** was 12.2% and that of the mixture of the other three isomers was 61.1%.

The synthetic (2S, 3S, 4R)-**I** was crystallized from water-methanol-ethyl acetate, m.p. 144~145°C (lit[4]. 144~146°C). The IR spectrum (in KBr disc) was the same as the natural material. The optical purity was confirmed by ORD measurements, synthetic: 230 nm (trough), $[\phi]=-234°$, natural: 230 nm (trough), $[\phi]=-234°$, C=1 in 1 N HCl at 16°C.

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