CHEMISTRY LETTERS, pp. 1155-1158, 1973. Published by the Chemical Society of Japan

# FRACTIONAL CRYSTALLIZATION OF A DIASTEREOMERIC DIPEPTIDE

Ryoichi KATAKAI, Kin-ichiro HOSHINO, Masanao OYA
Department of Industrial Chemistry, College of Technology,
University of Gunma, Tenjin-cho, Kiryu-shi 376
Fujio TODA, Keikichi UNO, and Yoshio IWAKURA
Department of Synthetic Chemistry, Faculty of Engineering,
The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113

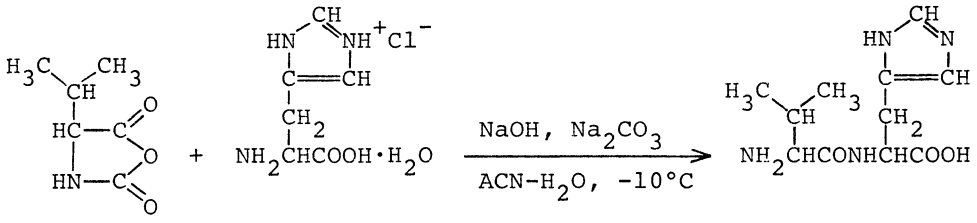
A successful fractional crystallization has been achieved on a diastereomeric dipeptide, DL-valyl-L-histidine to the D-L and L-L isomers. Resolution to the isomers with full optical activity is almost quantitative. Characterization of the dipeptide system by thin layer chromatography is also reported.

Separation of diastereomeric peptides has great importance from the viewpoint of the check of racemization in the peptide bond formation.[1] The separation of diastereomeric dipeptides has been achieved by thin layer [2-4] and column [5] chromatography. No separation of free dipeptide containing racemic amino acid residue by recrystallization has succeeded though the successful fractional crystallization has been sometimes carried out on the diastereomeric peptide derivatives with blocked terminal groups.[6,7]

In the course of the synthesis of some dipeptides containing the amino acids having polar side chains at the C-terminus, we have found that the sulfate of the diastereomeric dipeptides of valylhistidine shows a marked difference of the solubility in methanol, that is, L-valyl-L-histidine monosulfate is soluble in methanol but a diastereomeric isomer, D-valyl-L-histidine is insoluble in methanol. This finding led us to try the fractional crystallization of the dipeptide containing racemic amino acid residue, DL-valyl-L-histidine to the diastereomeric isomers.

DL-Valyl-L-histidine was prepared by the controlled reaction of DL-valine N-carboxy anhydride (NCA) with L-histidine monohydrochloride monohydrate in the

heterogeneous system of acetonitrile-water in 82% yield,[8] mp 181-182°C(decomp.), $[\alpha]_D$ +10.0°(c 2.0, water). Anal. Calcd for $C_{11}H_{18}N_4O_3 \cdot 2H_2O$: C,45.49; H,7.64; N,19.31%. Found: C,45.35; H,7.68; N,19.26%.




The dipeptide (10.1 g, 0.04 mol) was dissolved in 100 ml of methanol. Then concentrated sulfuric acid was dropped into the methanolic solution until no white precipitate became to appear. To this end point, the quantity of sulfuric acid added to the methanolic solution was equal (0.04 mol) to that of the dipeptide therein. The system was allowed to stand overnight at room temperature and the precipitate was collected and dried over $P_2O_5$. The product obtained as the first crop was dissolved in 25 ml of water. Fine needle crystals were obtained by addition of 200 ml of methanol to the aqueous solution. The purified product was identical with D-valyl-L-histidine monosulfate monohydrate, 11.1 g (75% yield). mp 202°C, $[\alpha]_D$ -34.0(c 2.0, water).
Anal. Calcd for $C_{11}H_{18}N_4O_3 \cdot H_2SO_4 \cdot H_2O$: C,35.66; H,5.99; N,15.34; S,8.43%.
Found: C,35.75; H,6.20; N,15.21; S,8.15%.

The filtrate was concentrated at reduced pressure, the resulting oil being crystallized by addition of diethyl ether. This solid was a mixture of L-valyl-L-histidine with a minor component of D-valyl-L-histidine. The latter was removed as an insoluble part in dissolving the mixture in methanol. After twice fractional crystallization, the insoluble D-L isomer was recovered as the second crop from the filtrate in 18% yield, which gives the total yield of 93% of D-valyl-L-histidine when the yield of the first crop is added. The L-L isomer was obtained in 87% yield as a white powder after isolation from the methanolic solution, mp 218°C, $[\alpha]_D$ +22.6°(c 2.0, water). Anal. Calcd for $C_{11}H_{18}N_4O_3 \cdot H_2SO_4 \cdot 2H_2O$: C,34.00; H,6.23; N,14.43%. Found: C,34.14; H,6.33; N,14.25%.

The resolved D-L and L-L isomers were demonstrated to be of full optical

activity by comparing the authentic samples synthesized by the controlled reaction of D- and L-valine NCA with L-histidine. Other dipeptides, L-DL, DL-D, and D-DL valylhistidine were also resolved to the diastereomeric isomers by the same fractional resolution as above. The results of the resolution and the optical rotation of the resolved isomers are listed in Table 1.

Though, in principle, the dipeptides containing DL-amino acid residue may be completely resolved to the diastereomeric isomers, there are a little loss of yield in the resolution, especially on the L-L and D-D isomers, perhaps because of higher solubility of these isomers in water in purification. The resolved diastereomeric isomers were isolated as the monosulfate which was demonstrated by titration with sodium hydroxide and was consistent with the fact of consuming sulfuric acid equal mol to the dipeptide in the resolution mentioned in above experimental details. Normally the peptide containing histidine residue may be present as a half salt of sulfuric acid. The present abnormal monosulfate of the dipeptides may play an important role in solubility of the D-L isomer though the clear explanation of the fact of resolution cannot be presented in this stage of study.

The dipeptide containing racemic amino acid residue failed to be fractionally resolved to the isomers as the salt of hydrochloride, acetate, or nitrate. Moreover other dipeptide system containing histidine at the C-terminus, e.g. DL-alanyl-L-histidine or DL-leucyl-L-histidine, could not be clearly resolved

Table 1. Results of resolution and optical rotation of the resolved diastereomeric isomers of valylhistidine

| Configuration of valylhistidine | Yield(%) in resolution to and optical rotation($[\alpha]_D^a$) of | | | |
|---|---|---|---|---|
| | D-L | L-D | L-L | D-D |
| | Yield, O.R. | Yield, O.R. | Yield, O.R. | Yield, O.R. |
| DL-L | 93 -34.0 | ———— | 87 +22.6 | ———— |
| L-DL | ———— | 92 +34.8 | 85 +21.5 | ———— |
| DL-D | ———— | 90 +33.9 | ———— | 88 -22.3 |
| D-DL | 92 -33.2 | ———— | ———— | 90 -21.8 |

[a] Optical rotation was measured at the concentration of 2.0 g/dl in water.

as the monosulfate. These facts suggest that the present successful fractional resolution of valylhistidine system is ascribed to cooperation of many factors including the conformation of the dipeptide, the bulkiness of the N-terminal amino acid side chain, the nature of the salt, and the imidazole side chain of histidine.

Since some dipeptides have been successfully resolved in chromatography,[2-5] thin layer chromatography was carried out on the resolved L-L and L-D valyl-histidine monosulfates and their free compounds with expectation of large separation of them. The solvent systems of pyridine-water, ethyl acetate-water-pyridine-acetic acid, butanol-acetic acid-water, and phenol-water were used as developer. In all developer systems, both the free dipeptides and the monosulfates could not be separated practically owing to high partition characteristics into water enhanced by the imidazole side chain of histidine residue.

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(Received August 24, 1973)