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**Note**

# Resolution of histidine diastereomers by gas chromatography

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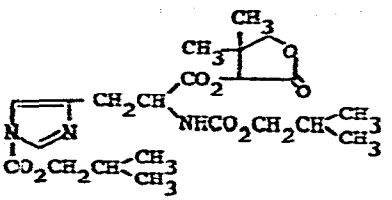
Although the gas chromatographic (GC) separation of diastereomeric histidine derivatives on a capillary column has recently been accomplished by König and coworkers[1], to our knowledge the separation of histidine diastereomers on a conventionally packed column with an optically inactive stationary phase has so far not been reported.

This paper describes a method for the separation and determination of enantiomeric histidines as their N-isobutyloxycarbonyl (isoBOC) L-(+)-pantoyl lactone esters (I). The derivatization procedure is based on N-isobutyloxycarbonylation in aqueous alkaline medium[2], followed by esterification with L-(+)-pantoyl lactone using N,N-carbonyldiimidazole (CDI) as a condensation reagent[3]. GC separation of the resulting diastereomers was carried out on a conventionally packed column.



(I)

## EXPERIMENTAL

### *Reagents*

Isobutyl chloroformate stabilized with calcium carbonate was obtained from Eastman-Kodak (Rochester, N.Y., U.S.A.) and used without further purification. L-(+)-Pantoyl lactone of *ca.* 99.5% optical purity was obtained from Tokyo Kasei (Tokyo, Japan) and dried under vacuum overnight. CDI was obtained from Merck (Darmstadt, G.F.R.) and stored in a desiccator. DL-Histidine monohydrochloride was obtained from Nakarai Chemicals (Kyoto, Japan), and D-histidine monohydrochloride, L-histidine monohydrochloride and arachidic acid as an internal standard from Sigma (St. Louis, Mo., U.S.A.). Triethylamine was obtained from Nakarai Chemicals and dried over sodium hydroxide. All other reagents and solvents were reagent grade purity and used as received from commercial sources.

*Derivatization*

N-Isobutyloxycarbonylation of histidine was carried out by the method previously reported[2]. To the ethereal extracts containing N-isoBOC-histidine was added 10 $\mu$g of arachidic acid and the solvent was evaporated to dryness. After addition to the residue of 0.1 ml of freshly prepared 1.0 *M* CDI in dichloromethane, the mixture was left to stand for 10 min at room temperature and subsequently 0.1 ml of a dichloromethane solution containing 50% triethylamine and 30% L-(+)-pantoyl lactone was added. After standing for 10 min at 40°, 4 ml of water saturated with sodium chloride was added and then the resulting N-isoBOC-L-(+)-pantoyl lactone ester of histidine was extracted three times with 3 ml of *n*-hexane. The combined *n*-hexane extracts were evaporated to dryness and the residue was dissolved in 0.1 ml of ethyl acetate. A 2–4 $\mu$l volume of the resulting solution was injected on to the gas chromatograph.

*Gas chromatography*

A Shimadzu Model 4CM gas chromatograph equipped with a hydrogen flame ionization detector, and an on-column injection port was used. The operating conditions are given in Fig. 1. The liquid phase, OV-17, and the support, Uniport HP (100–120 mesh) were purchased from Gasukuro Kogyo (Tokyo, Japan). The glass column (1 m) and quartz-wool plugs placed in each end of the column used throughout this work were silanized with dimethyldichlorosilane vapour. The column packing, 3% OV-17 on Uniport HP, was prepared by the "solution coating" technique[4].

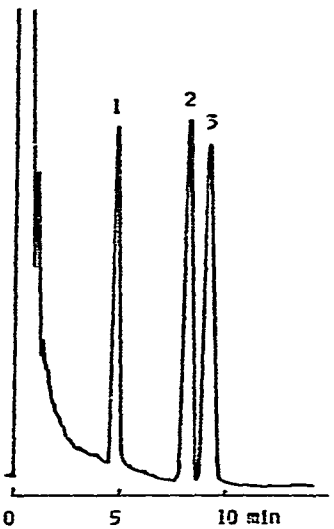



Fig. 1. Gas chromatogram of N-isoBOC-DL-histidine L-(+)-pantoyl lactone esters. Conditions: column, 3% OV-17 on Uniport HP (100–120 mesh), 1 m × 3 mm I.D., glass; column temperature, 280°; nitrogen flow-rate, 40 ml/min. Peaks: 1 = arachidic acid (internal standard); 2 = D-histidine derivative; 3 = L-histidine derivative.

## RESULTS AND DISCUSSION

The two-step process involving N-isobutyloxycarbonylation and esterification was used to prepare the derivatives. N-Isobutyloxycarbonylation was performed in the first step exactly according to our previous method[2]. This reaction is extremely useful because it proceeds simply and rapidly in aqueous alkaline medium at room temperature. Subsequently, esterification for the introduction of a second chirality into N-isoBOC-histidine with an optically active alcohol using CDI as a condensation reagent was carried out with modifications according to the method of Ko and Royer[3]. From the results of preliminary experiments on separation and GC properties, L-(+)-pantoyl lactone was found to be the most suitable as a resolving reagent among various optically active alcohols tested. This alcohol is commercially available at relatively low cost.

The structures of the derivatives of D- and L-histidine prepared by the procedure described above were elucidated by the use of a Shimadzu LKB 9000 gas chromatograph–mass spectrometer. Each of derivatives gave a molecular ion peak with a *m/e* value of 467 and the same fragment pattern, and this suggests that they have the structure of (I).

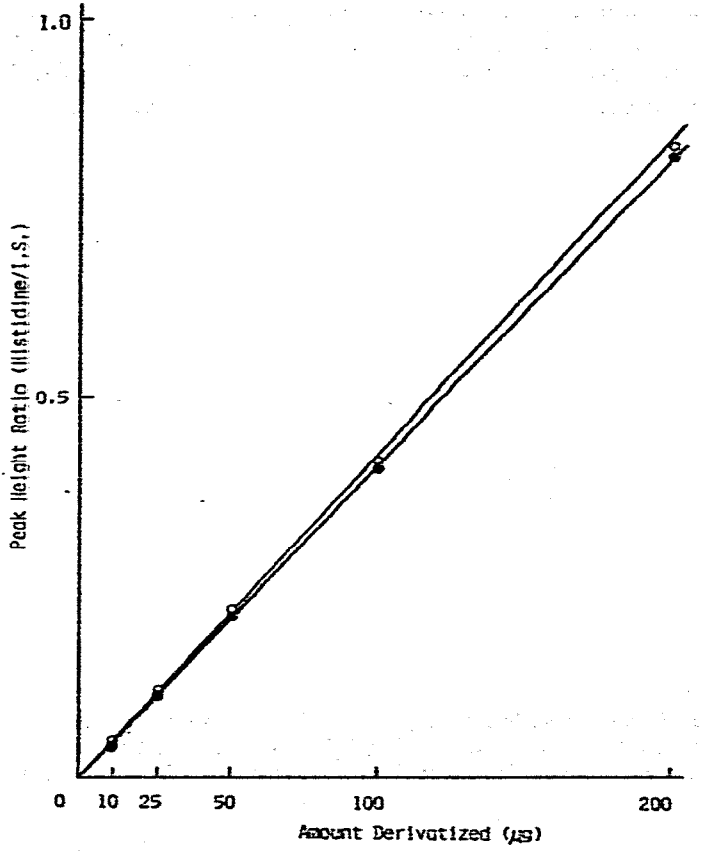



Fig. 2. Calibration curves for enantiomeric histidine. ○, D-Histidine; ●, L-histidine; internal standard, arachidic acid (10 μg).

For the separation of the diastereomeric histidine derivatives a 1-m column packed with 3% OV-17 on Uniport HP was found to be sufficient. The chromatogram of the histidine derivatives is illustrated in Fig. 1, showing the separation. The retention time of the D-L compound is shorter than that of the L-L diastereomer, and each gave a single and symmetrical peak. Throughout this derivatization procedure no racemization was observed. The separation factor, which was calculated by the method of Nambara and co-workers[5], is 1.00, and this indicates that the separation of the histidine diastereomers was complete. The linearity of the calibration curve for each enantiomer in the range studied (10–200 $\mu$g) was found to be satisfactory (Fig. 2). In order to examine the quantitative reliability of this method, mixtures with various known proportions of D- and L-histidine were derivatized and analysed. The results are shown in Fig. 3. The peak height ratios of N-isoBOC-L-(+)-pantoyl lactone esters of D- and L-histidine show a linear relationship with the ratio of enantiomers present. Therefore, it can be seen that the method would be suitable for the determination of enantiomeric percentages of histidine in biological samples.

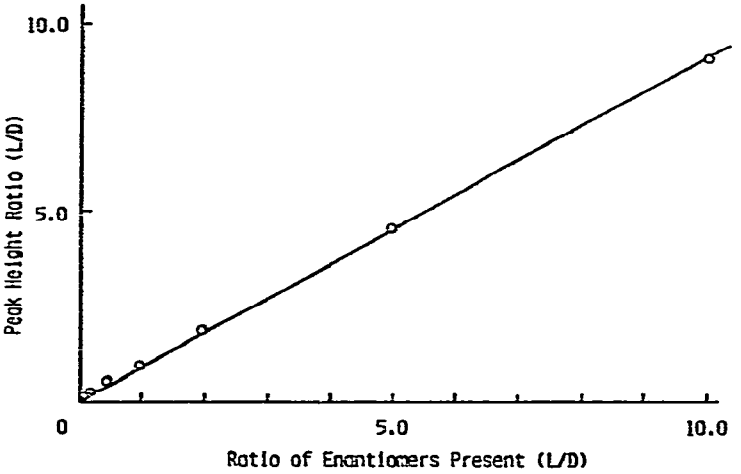



Fig. 3. Relationship between peak height ratio and enatiomeric composition of mixtures of D- and L-histidine.

This method is simple and convenient because a conventionally packed column with a thermostable phase such as OV-17 can be used, and derivatization can be performed within 40 min without requiring an elaborate procedure. Moreover, the resulting derivatives are very stable towards moisture.

Application of the technique to other amino acids is in progress.

## REFERENCES

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