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Note

Separation of L- and D-amino acids as diastereomeric derivatives by high-performance liquid chromatography

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In a preliminary communication[1], we reported an effective chromatographic separation of four racemic amino acids as the diastereomeric mixture of N-*d*-10-camphorsulphonyl *p*-nitrobenzoate by high-performance liquid chromatography (HPLC) using a silica gel (MicroPak Si-5) column packing and 1.5% isopropanol in isooctane as the eluting solvent. The N-*d*-10-camphorsulphonyl moiety served to introduce an additional asymmetric centre and the *p*-nitrobenzyl group as a chromophore for detection.

We now report the application of the method to the amino acids methionine, glutamic acid, tryptophan, tyrosine, isoleucine, leucine, phenylalanine and alanine.

EXPERIMENTAL

Apparatus and conditions

An FLC 350 high-performance liquid chromatograph (JASCO) with gradient capability and a UV-254 detector monitoring at 253.7 nm were used. The column employed was a stainless-steel tube, 25 cm × 2.2 mm I.D., slurry-packed with microporous chemically bonded silica gel (Varian MicroPak-NH_2, average particle size 10 μm) and operated at ambient temperature. The flow-rate of the mobile phase was adjusted using pressures of 20–50 kg/cm^2.

Reagents and chemicals

All solvents were of reagent grade and were distilled prior to use. Amino acids were obtained from Katayama (Osaka, Japan), while *d*-10-camphorsulphonyl chloride was prepared from the corresponding acid[2].

Preparation of amino acid derivatives

A 30-ml volume of a solution of 2.0 mmole of *d*-10-camphorsulphonyl chloride in anhydrous diethyl ether was added dropwise to a solution of 1.0 mmole of amino acid in 10 ml of diethyl ether plus 20 ml of 1 *N* sodium hydroxide solution with vigorous stirring at 0°. Stirring was subsequently continued at room temperature for 3 h. The aqueous layer was separated from the ethereal layer, washed with twice diethyl ether, acidified with concentrated hydrochloric acid and then extracted with diethyl ether. The ethereal solution was dried over anhydrous sodium sulphate and evapo-

rated to dryness. The residue was dissolved in 10 ml of N,N-dimethylformamide, then one drop of trimethylamine and 1.1 mmole of *p*-nitrobenzyl bromide were added. The reaction mixture was heated at 55° for 2 h, diluted with 40 ml of chloroform, washed with water, dried over anhydrous sodium sulphate and then evaporated to dryness to obtain the N-*d*-10-camphorsulphonyl *p*-nitrobenzoate of the amino acid.

Unless otherwise stated, a chloroform solution of the diastereomeric mixture of the derivatives of DL-amino acids was used for HPLC.

RESULTS AND DISCUSSION

We investigated the separation of DL-amino acid derivatives with dichloromethane as the eluting solvent. The purification has to be carried out carefully, in order to obtain constant retention times of the amino acid derivatives. The purification procedure was as follows: washed with 5% hydrochloric acid, 5% potassium carbonate solution and then water (five times each), dried over anhydrous sodium sulphate, distilled to collect the fraction of b.p. 39°, and used immediately. The derivatives of D- and L-alanine, -glutamic acid, -methionine and -phenylalanine were separated completely, as shown in Fig. 1. However, the long retention times of each of the amino acid derivatives were not convenient for our purpose.

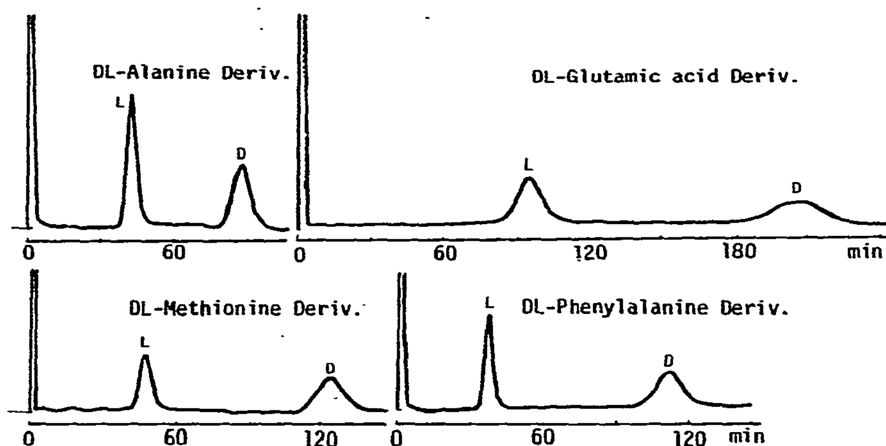
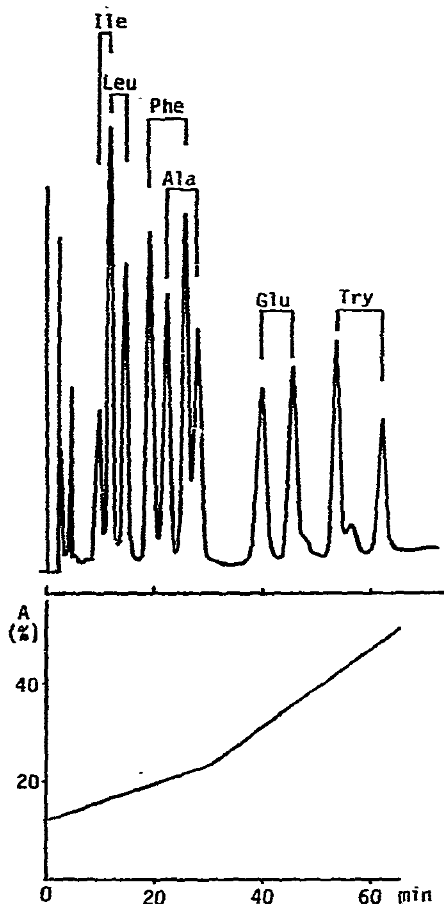


Fig. 1. Chromatograms of the diastereomers of N-*d*-10-camphorsulphonyl *p*-nitrobenzyl amino acids. Flow-rate, 0.4 ml/min; column, MicroPak-NH_2; eluent, dichloromethane.

To shorten the analysis time, a variety of solvents and gradient systems were investigated and chromatograms of mixtures of some DL-amino acid derivatives and the corresponding gradient diagrams are illustrated in Figs. 2 and 3. Excellent separations of all amino acid derivatives were observed. In order to identify the peaks, optically enriched amino acid derivatives were prepared under the same reaction condition as described above. No racemization was observed during the preparation of the derivatives, because each derivative showed a single peak in the chromatogram.

Table I shows typical retention times obtained with two solvent systems consisting of isooctane plus dichloromethane in different proportions, each containing 5%



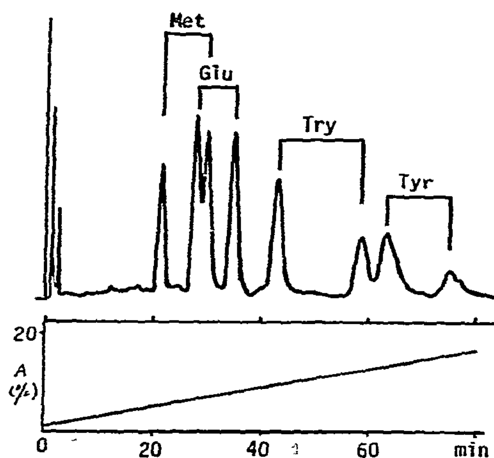


Fig. 2. Chromatogram and gradient diagram of the diastereomers of N-*d*-10-camphorsulphonyl *p*-nitrobenzyl amino acids. Flow-rate, 0.5 ml/min; column, MicroPak-NH_2. Eluent: A, isooctane–dichloromethane–isopropanol (70:15:15); B, isooctane–dichloromethane (90:10).

Fig. 3. Chromatogram and gradient diagram of N-*d*-10-camphorsulphonyl *p*-nitrobenzyl amino acids. Flow-rate, 0.7 ml/min; column, MicroPak-NH_2. Eluent: A, isooctane–dichloromethane–isopropanol (35:50:15); B, isooctane–dichloromethane (50:50).

TABLE I

RETENTION TIMES (min) OF D- AND L-AMINO ACID DERIVATIVES

Eluent: A, isooctane–dichloromethane–isopropanol (79:16:5); B, isooctane–dichloromethane–isopropanol (63:32:5). Flow-rate, 0.4 ml/min. Column, MicroPak-NH_2.

Amino acid	*Eluent A*			*Eluent B*		
	L	D	D/L	L	D	D/L
Leucine	3.9	4.4	1.1	2.7	2.8	1.0
Isoleucine	4.4	5.0	1.1	2.9	3.1	1.1
Phenylalanine	6.2	8.5	1.4	3.3	4.1	1.2
Methionine	7.4	10.0	1.4	3.6	4.6	1.3
Alanine	7.2	9.3	1.3	3.7	4.4	1.2
Glutamic acid	12.8	16.8	1.3	4.2	5.2	1.2
Tryptophan	29.2	49.6	1.7	9.0	14.9	1.7
Tyrosine	33.2	47.2	1.4	11.6	16.2	1.4

of isopropanol. The results show that the variation of the ratio of isooctane to dichloromethane affects the absolute retention time of each amino acid derivative but not the relative retention times of corresponding D- and L-amino acids.

Of the amino acids tested, tryptophan and phenylalanine were detectable with the UV-254 detector without introducing the *p*-nitrobenzyl moiety as a chromophore. The chromatogram of the methyl ester of N-*d*-10-camphorsulphonyl phenylalanine is shown in Fig. 4. Compared with the chromatogram of the *p*-nitrobenzoate of the corresponding derivative, the methyl ester seems to be more efficient from the point of view of the separation of enantiomers, the retention times and the preparation of the derivative.

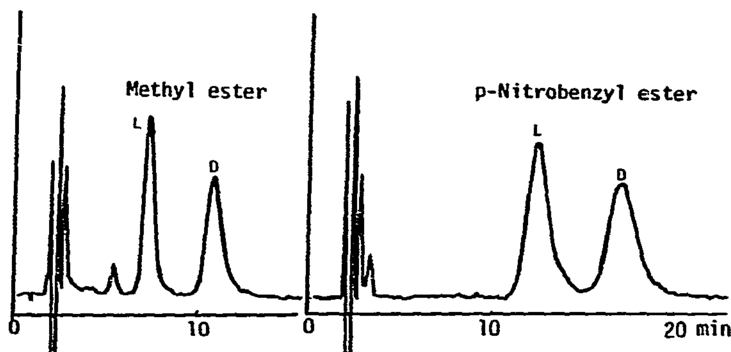


Fig. 4. Chromatograms of the methyl ester and *p*-nitrobenzyl ester of the diastereomers of N-*d*-10-camphorsulphonyl phenylalanine. Flow-rate, 0.4 ml/min; column, MicroPak-NH_2; eluent, isooctane–dichloromethane–isopropanol (87:8:5).

CONCLUSIONS

For all of the amino acid derivatives tested ,the retention times of the L-amino acid derivatives were consistently shorter than those of the corresponding D-amino acid derivatives. Consequently, it could possibly be assumed tentatively that there is a correlation between retention time and absolute configuration, which might be useful for the assignment of the absolute configuration of new amino acids.

REFERENCES

1 H. Furukawa, E. Sakakibara, A. Kamei and K. Ito, *Chem. Pharm. Bull.*, 23 (1975) 1625.
2 P. D. Bartlett and L. H. Knox, *Org. Syn.*, 45 (1956) 45.