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Short Communication

Separation of aspartame and its precursor stereoisomers by chiral chromatography

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ABSTRACT

The four stereoisomers of the protected dipeptide LL-, LD-, DL- and DD-(Z)-Asp(β-Bzl)–Phe-OCH_3, an aspartame precursor, were successfully separated by high-performance liquid chromatography with a Chiralcel OD column. Of the four stereoisomers of aspartame, LL-, LD-, DL- and DD-Asp–Phe-OCH_3, two pairs of diastereoisomers, DD/DL and LL/LD, cannot be separated by thin-layer chromatography on a CHIRALPLATE, whereas two other pairs of diastereoisomers, DD/LD and LL/DL, and two pair of enantiomers, DD/LL and DL/LD, could be well separated.

INTRODUCTION

Aspartame is a dipeptide (L-Asp–L-Phe-OCH_3) widely used in foods because of its sweet taste, and is better known as Nutrasweet [1–3]. The synthesis of the dipeptide has been developed in several ways, either by chemical methods or chemoenzymatic methods [4–8]. DL-, LD- and DD-(Asp–Phe-OCH_3) are possible byproducts, particularly in chemical methods or by enzymatic condensation with D,L-amino acid derivatives as substrates. In order to examine the stereochemical purity of aspartame and its precursors, [(*Z*)-Asp(β-Bzl)–Phe-OCH_3], chiral chromatography was used to separate the stereoisomers of the peptides.

EXPERIMENTAL

L-Amino acids were purchased from Kyowa Fermentation (Tokyo, Japan) and D-amino acids from Sigma (St. Louis, MO, U.S.A.). All solvents were obtained from Alps Chemical (Taiwan). Chiral Pirkle-type 1-A (25 cm × 4.6 mm I.D.) and Pirkle-concept Sumipax OA-1000, -1100, -2200, -3000 and -4000 chiral columns (15 cm × 4.6 mm I.D.; d_p = 5 μm) were obtained from Regis Chemical (Morton

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Grove, IL, U.S.A.) and a Chiralcel OD column (25 cm × 4.6 mm I.D.) was from Daicel Chemical Industries (Japan). A CHIRALPLATE was purchased from Macherey, Nagel & Co. (Düren, Germany).

DD, DL, LL and LD protected dipeptide stereoisomers [(*Z*)-Asp(β-Bzl)–Phe-OCH_3] were synthesized by the dicyclohexylcarbodiimide coupling method and their purity was checked on by thin-layer chromatography (TLC) on silica gel Type 60 (E. Merck, Darmstadt, Germany) by the chlorine–tolidine method [9]. The four DD, LL, DL and LD stereoisomers of aspartame (Asp–Phe-OCH_3) were prepared from the protected dipeptides by hydrogenation.

Separation of the four DD, DL, LL and LD protected dipeptide stereoisomers by chiral high-performance liquid chromatography (HPLC)

An HPLC system from Waters Assoc. (Milford, MA, U.S.A.) was used for the analytical separations, consisting of an M6000A solvent delivery unit and a U6K universal liquid chromatograph injector, coupled to an M450 variable-wavelength UV spectrophotometer and a SIC chromatocorder 12 integrator (System Instruments, Japan). The diastereoisomers of the protected dipeptide were separated on chiral columns using 2-propanol in *n*-hexane as the mobile phase at room temperature and detected by UV spectrophotometry at 254 nm.

Separation of the four DD, DL, LL and LD aspartame stereoisomers by chiral TLC

The four stereoisomers of aspartame were separated on a CHIRALPLATE with methanol–water–acetonitrile (50:50:200, v/v/v) as the developing solvent and detected using ninhydrin solution.

RESULTS AND DISCUSSION

Most dipeptides usually have four different stereoisomers. From previous studies [10,11], protected diastereoisomers such as DD/DL and LL/LD could be separated by normal- or reversed-phase HPLC, while protected enantiomers such as DD/LL and DL/LD were separated by chiral HPLC [12]. However, the four stereoisomers of the protected dipeptide were never completely resolved simultaneously by a single separation method. As shown in Fig. 1 and Table I, the four stereoisomers of the aspartame precursor, DD-, DL-, LL- and LD-[(*Z*)-Asp(β-Bzl)–Phe-OCH_3] were completely separated by the Chiralcel OD column. However, for other types of chiral columns such as Pirkle-type 1-A, Sumipax OA-1000, -1100, -2200, -3000 and -4000, the four stereoisomers cannot be well separated (Fig. 2). In this preliminary studies, a Chiralcel OD column, which is an inclusion-type column and possesses a complicated mechanism for the separation of stereoisomers [13], is much better for the separation of the four stereoisomers of the aspartame precursor than Pirkle-type columns.

Recently, the two pairs of enantiomers of aspartame were separated by HPLC with immobilized α-chymotrypsin as the stationary phase [14]. Although the enantioselectivities for DL/LD and DD/LL enantiomers were very high, the four stereoisomers were not completely separated under one set of separation conditions. The CHIRALPLATE was developed for the separation of racemic amino acids and has also been used to resolve stereoisomers of dipeptides [15,16]. The resolution of the

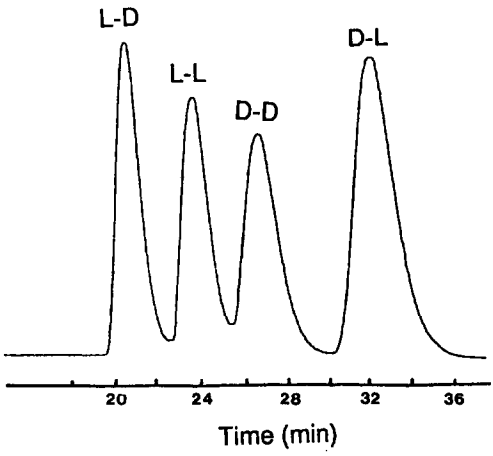

Fig. 1. HPLC profile of the four stereoisomers of (*Z*)-Asp(β-Bzl)–Phe-OCH_3 (20 μg for each dipeptide) on a Chiralcel OD column. Elution with 2-propanol-*n*-hexane (1:1) at a flow-rate of 0.5 ml/min at room temperature and UV detection at 254 nm, a.u.f.s. 2.0.

four stereoisomers of aspartame were conducted with a CHIRALPLATE. According to the results shown in Fig. 3, two pairs of diastereomers, DD/DL and LL/LD, cannot be separated; whereas two other pairs of diastereomers, DD/LD and LL/DL and two pairs of enantiomers, DD/LL and DL/LD, could be well separated. Based on previous reports, the separation of stereoisomers of dipeptides on a CHIRALPLATE depends on the chirality of the N-terminal amino acid residues. Generally, stereoisomers of dipeptides which have different chirality in the N-terminal amino acid residues could be resolved on a CHIRALPLATE and dipeptides containing L-amino acid residues at the N-terminus have higher mobilities than those containing D-amino acid residues at the N-terminus. These phenomena are similar to those with free amino acids. However, in this instance, DD- and DL-aspartame (R_F = 0.62) have higher mobilities than LL- and LD-aspartame (R_F = 0.50).

TABLE I

CAPACITY FACTORS (k'), SEPARATION FACTORS (α) AND RESOLUTION FACTORS (R_s) OF THE FOUR STEREOISOMERS OF THE PROTECTED DIPEPTIDE WITH DIFFERENT CHIRAL COLUMNS

Stereoisomer	Chiralcel OD column			Pirkle-type 1-A column			OA-1100 column		
	k'	α	R_s	k'	α	R_s	k'	α	R_s
LL	2.56	1.19	0.87	1.75	1.03	0.40	2.72	1.07	0.73
DD	3.06			1.81			2.55		
LD	2.07	2.07	2.61	2.03	1.00	–	3.04	1.00	–
DL	3.92			2.03			3.03		

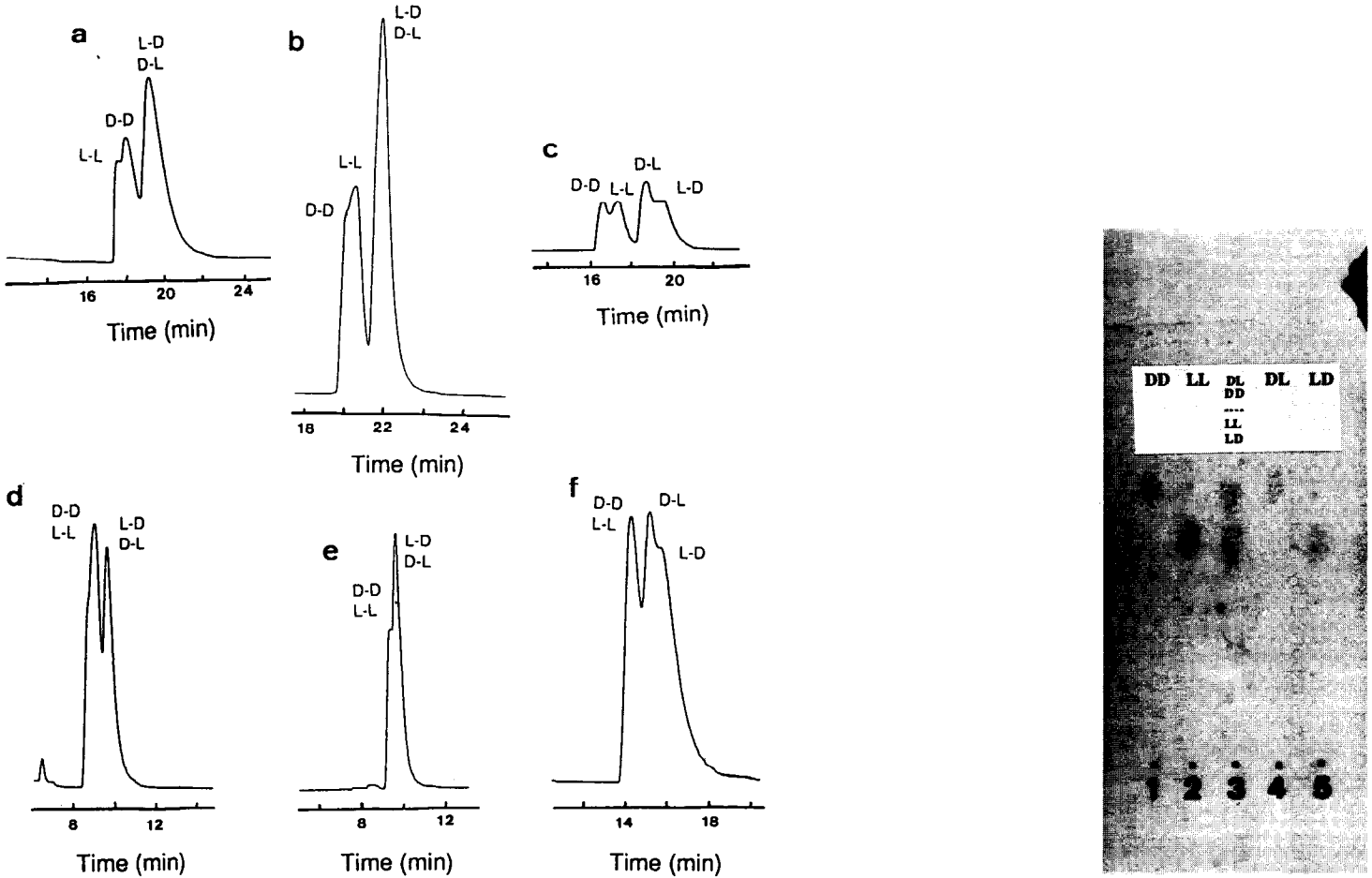

Fig. 2. HPLC profiles of the four stereoisomers of (*Z*)-Asp(β-Bzl)–Phe-OCH_3 (20 μg for each dipeptide) on (a) Pirkle-type 1-A, (b) Sumipax OA-1000, (c) OA-1100, (d) OA-2200, (e) OA-3000 and (f) OA-4000 columns. The Pirkle-type 1-A column was eluted isocratically with 2-propanol–*n*-hexane (1:5) at a flow-rate of 1 ml/min and the other columns with 2-propanol–*n*-hexane (1:9) at a flow-rate of 0.5 ml/min (0.3 ml/min for OA-3000) at room temperature with UV detection at 254 nm, a.u.f.s. 2.0.

Fig. 3. TLC of the four stereoisomers of aspartame on a CHIRALPLATE. (1) DD; (2) LL; (3) mixture of four stereoisomers; (4) DL; (5) LD. Developing system and

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