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Synthesis and Characteristics of an Aspartame Analogue, *L*-Asparaginyl *L*-3-Phenyllactic Acid Methyl Ester

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Abstract An aspartame analogue, *L*-asparaginyl *L*-3-phenyllactic acid methyl ester was synthesized with aspartic acid replaced by asparagine and peptide bond replaced by ester bond. The aspartic acid of aspartame could be replaced by asparagine as reported in the literature. In this analogue, the hydrogen of amide group could still form a hydrogen bond with the oxygen of ester bond and the ester bond was isosteric with peptide bond. However, the product was not sweet, showing that the peptide bond could not be replaced by ester bond. The peptide C-N bond behaves as a double bond that is not free to rotate and the C, O, N and H atoms are in the same plane. The replacement of peptide bond by ester bond destroyed the unique conformation of peptide bond, resulting in the loss of sweet taste.

Key words artificial sweetener; aspartame derivative; peptide bond

Aspartame (*L*-aspartyl *L*-phenylalanine methyl ester) is an artificial sweetener as shown in Fig.1 (A) [1]. Studies on its structure and function showed that its N-terminal *L*-aspartyl residue could only be replaced by aminomalonyl [2] or *L*-asparaginyl [3] residue. When its peptide bond was replaced by an ester bond [Fig. 1(B)] or the hydrogen of amide in the peptide bond replaced by a methyl group [Fig. 1(C)], its sweetness was lost [4]. According to the crystal structure of aspartame, between the β-carboxyl oxygen of aspartic acid and the amide hydrogen of peptide bond there is a hydrogen bond [5] which is not present when the peptide bond is replaced by an ester bond.

Here, we prepared an aspartame analogue, *L*-asparaginyl *L*-3-phenyllactic acid methyl ester [aspartame ester, Fig. 1(D)]. In comparison with aspartame, this analogue has an ester bond instead of peptide bond and an N-terminal asparagine instead of aspartic acid. The amide hydrogen of asparagine can still form a hydrogen bond with the oxygen of ester bond.

Materials and Methods

Materials

L-3-phenyllactic acid, N-hydroxysuccinimide (HOSu) and 4-diaminopyridine (DAMP) were products of Fluka Co.. Trifluoroacetic acid (TFA) and methanol were products of Merck Co.. Tertiary butyloxycarbonyl *L*-asparagine (Boc-*L*-Asn) was from Peptide Institute Inc. in Japan, N-methylmorpholine and dicyclohexylcarbodiimide (DCC) from Tokyo Kasei Kogyo Co., acetonitrile from ABI, and tetrahydrofuran, ethyl acetate and isopropyl ether from factories in Shanghai.

Peptide synthesis

The synthesis of *L*-asparaginyl *L*-3-phenyllactic acid methyl ester is shown in Fig. 2.

TLC analysis

HPTLC F254 (Merck) plate was used. Spots were developed by pyridine/acetic acid/water/butyl acetate/isopropanol (12:3:4.5:70:28) and examined at 254 nm.

HPLC purification

Waters 2487 workstation with Beckman RP C18 column (10 mm×250 mm) was used, sample volume: 0.5 ml, flow rate: 2 ml/min, wavelength 254 nm, buffer A: 0.1% TFA, buffer B: 0.8% TFA/70% acetonitrile, elution

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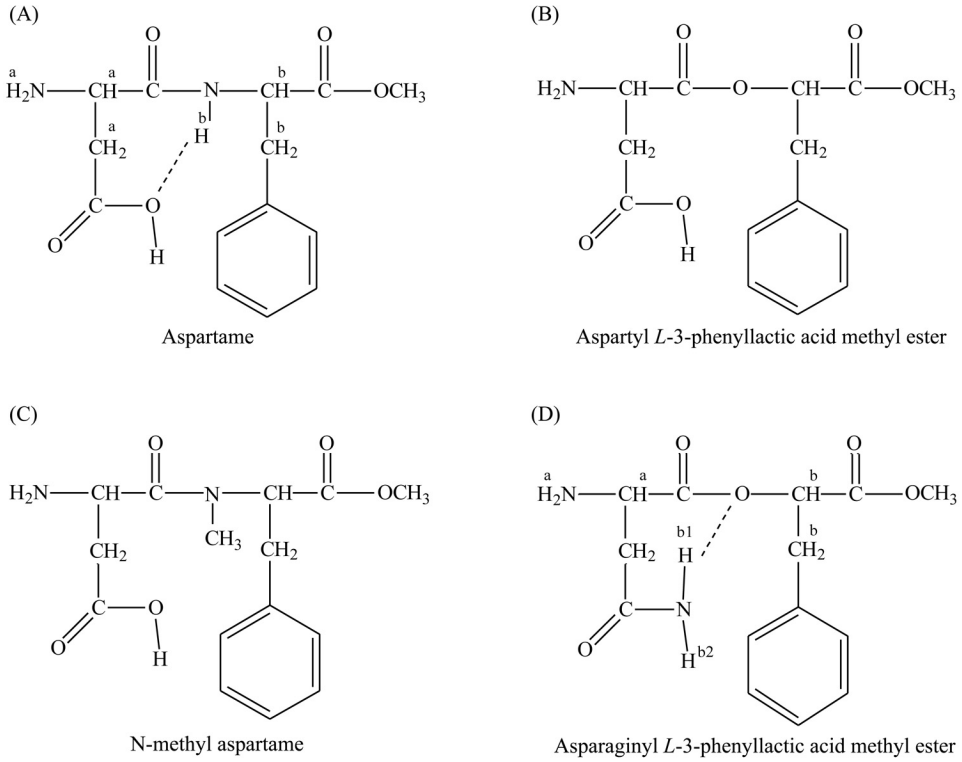

Fig. 1 Aspartame and its analogues

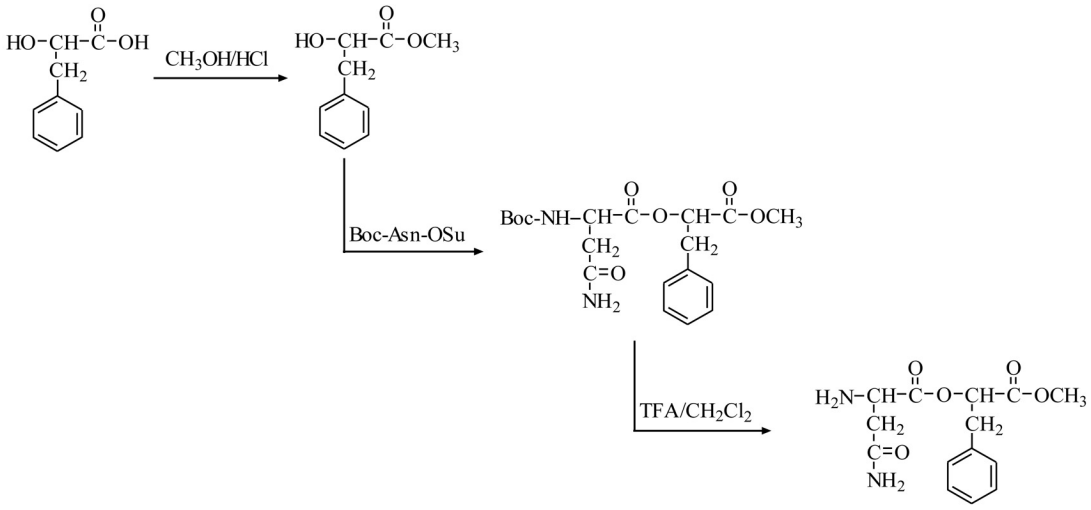

Fig. 2 Synthesis of *L*-asparaginyl *L*-3-phenyllactic acid methyl ester

gradient: 0%–100% buffer B in 30 min.

Mass spectroscopy

Finnigan electrospray ion trap mass spectroscopy was used.

NMR analysis

Bruker AV500 NMR instrument was used. The sample was dissolved in 0.5 ml water containing 10% D_2O and the pH was adjusted to 5.1 with phosphoric acid.

Assay of sweetness

2% sample solution and 2% sucrose solution were tested by 2 volunteers.

Results

Synthesis of *L*-3-phenyllactic acid methyl ester

1 g (6.05 mmol) *L*-3-phenyllactic acid was dissolved in 10 ml methanol, saturated with dry HCl in ice bath and stored overnight. The reaction was repeated. The solution was evacuated. The residue was recrystallized in tetrahydrofuran/petroleum ether (2:3) and dried in vacuo. 0.3 g crystalline product was obtained, mp 40–42 °C, R_f in TLC 0.72, yield 25%.

Synthesis of Boc-*L*-Asn N-hydroxysuccinimide (Boc-Asn-Osu) [6]

4.64 g (20 mmol) Boc-*L*-Asn and 2.3 g (20 mmol) N-hydroxysuccinimide (HOSu) were dissolved in 40 ml acetonitrile and cooled in ice bath. 4.12 g (20 mmole) DCC dissolved in 10 ml acetonitrile was added dropwise, stirred for 2 h and stored at 4 °C for 48 h. The viscous solution was filtered and Boc-Asn-OSu was crystallized by adding isopropyl ether. 3.64 g product was obtained, yield 55%, R_f in TLC 0.67.

Synthesis of *L*-asparaginyl *L*-3-phenyllactic acid methyl ester

0.5 g (2.79 mmol) *L*-3-phenyllactic acid methyl ester and 1.38 g (4.18 mmol) Boc-Asn-OSu were dissolved in 15 ml acetonitrile. 0.3 ml N-methylmorpholine was added to adjust the pH to 8. 0.2 g (1.67 mmol) DMAP was added and the reaction mixture was stirred overnight at room temperature. After evacuation, the residue was dissolved in 35 ml ethyl acetate, washed 4 times with 1% potassium carbonate and with saturated sodium chloride solution to neutral pH. The solution was dried overnight with anhydrous sodium sulfate. 0.78 g yellowish oil product (yield 71%) was obtained. The product was deblocked in 10 ml 50% TFA in methylene dichloride at 25 °C for 0.5 h. After evacuation, an oily product of *L*-asparaginyl *L*-3-phenyllactic acid methyl ester was obtained. It was purified by silica gel chromatography using pyridine/acetic acid/water/butyl acetate/isopropanol (12:3:4.5:70:28) as eluting solution. Fractions with TLC R_f of 0.33 were pooled and evacuated to dryness. The residue was dissolved in 30 ml 1 N acetic acid and lyophilized. After preparative HPLC purification, 11.2 mg aspartame ester was obtained. The product was hygroscopic white powder with TLC R_f 0.33 and MS (ESI) *m/z* 294.0 (theoretical value 294.12).

NMR analysis

The ^{1}H-NMR spectrum of aspartame and assignment of hydrogen are shown in Fig. 3. In the NOESY spectrum of aspartame (Fig. 4), there is a cross peak of CHa and NHb, indicating that the hydrogen bond between NHb and

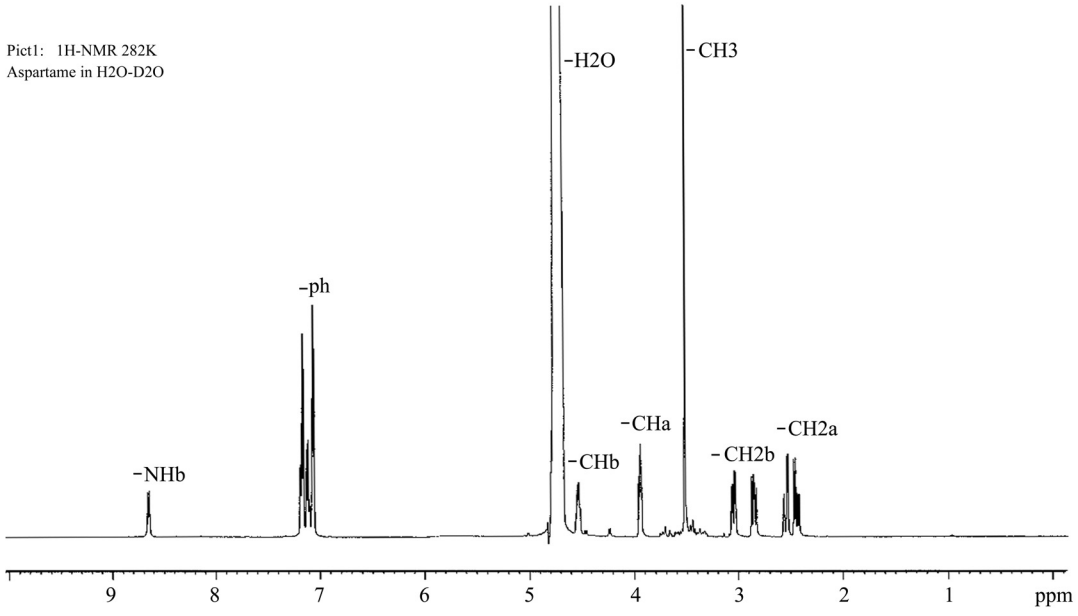

Fig. 3 **^{1}H-NMR spectral assignment for aspartame**

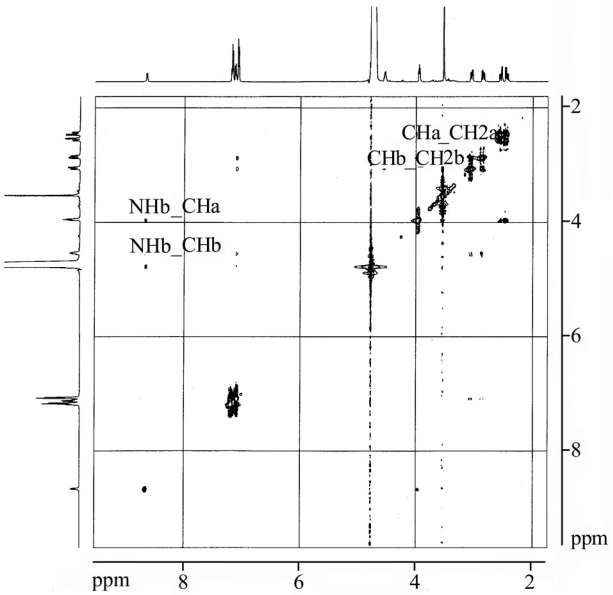

Fig. 4 The NOESY spectrum of aspartame

oxygen decreases the distance between CHa and NHb. It is in accord with the crystal structure of aspartame, that between the β-carboxyl oxygen of aspartic acid and the amide hydrogen of peptide bond there is a hydrogen bond [5].

The ^{1}H-NMR spectrum of aspartame ester analogue is shown in Fig. 5. Normally NHb1 and NHb2 are indistinguishable, but in aspartame ester analogue there is a difference of 0.66 ppm between the chemical shifts of NHb1 and NHb2, indicating that the formation of hydrogen bond increases the deshielding effect of oxygen and nitrogen on hydrogen. The presence of hydrogen bond was supported by the NOESY spectrum (Fig. 6). There is a cross peak of CH2a and NHb1, representing the NOE

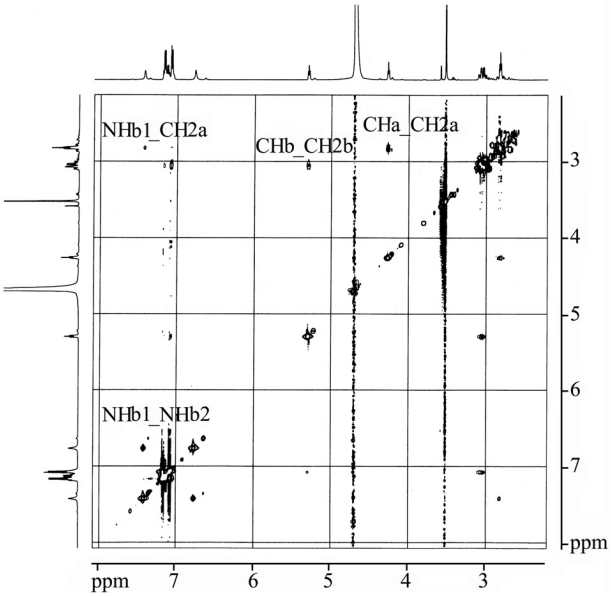

Fig. 6 The NOESY spectrum of *L*-asparaginyl *L*-3-phenyllactic acid methyl ester

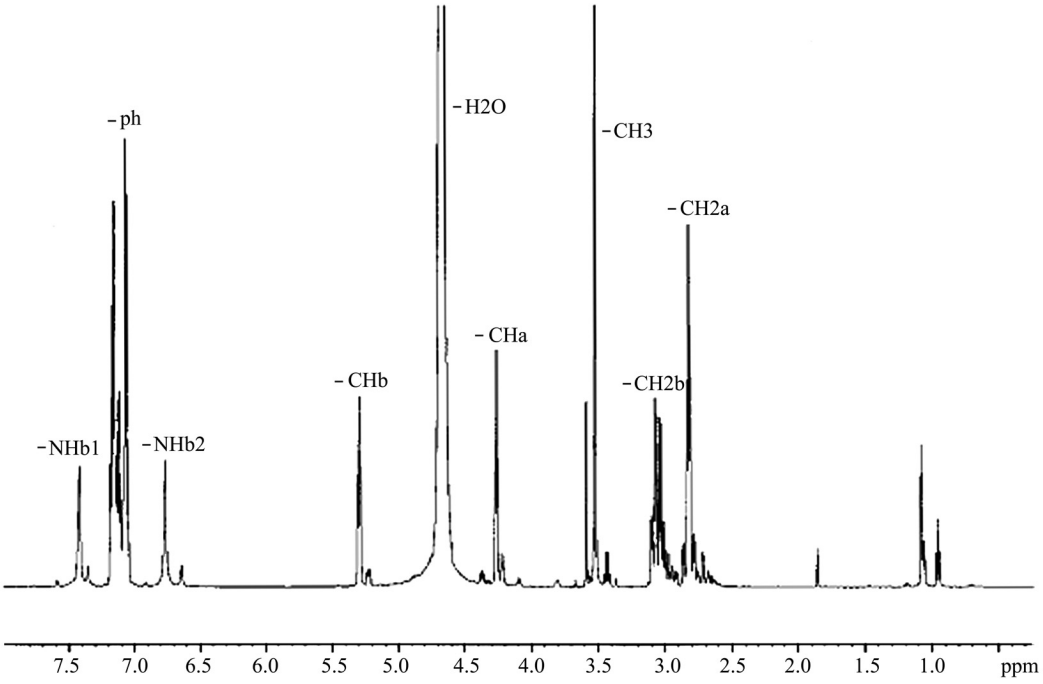

Fig. 5 ^{1}H-NMR spectral assignment for *L*-asparaginyl *L*-3-phenyllactic acid methyl ester

effect between them.

Assay of sweetness

Aspartame ester was tasted by 2 volunteers and no sweet taste was found.

Discussion

Since aspartame was discovered in 1965, a large number of analogues have been prepared to study its mechanism of action and develop better sweeteners [7–9]. Murray Goodman and his coworkers modified the peptide bond and studied its effect on the sweet taste [4]. When peptide bond was replaced by an ester bond or methylated, the product was no longer sweet. According to the crystal structure of aspartame [5], there is a hydrogen bond between the β-carboxyl group oxygen of Asp and the amide hydrogen of peptide bond, indicating that the hydrogen bond is essential for the sweetness. It was known that Asp could be replaced by Asn. In the present study, we prepared an aspartame analogue by replacing Asp with Asn and the peptide bond with ester bond. In this analogue, the amide group hydrogen of Asn could form a hydrogen bond with the oxygen of ester bond; in addition ester bond is isosteric with peptide bond. The presence of the hydrogen bond was confirmed by NMR analysis. However, in spite of the presence of hydrogen bond, the product still had no sweet taste, showing that the peptide bond could not be replaced by ester bond, though they are isosteric. In peptide bond, the C-N bond behaves as a double bond which is not free to rotate and the C, O, N and H atoms are in the same plane. The replacement of peptide bond by ester bond destroys the unique conformation of peptide bond, resulting in the loss of sweet taste.

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