Synthesis and Sweetness Characteristics of L-Aspartyl-D-Alanine Fenchyl Esters

Yoshifumi Yuasa,* Akira Nagakura, and Haruki Tsuruta

Technical and Engineering Department, Fine Chemical Division, Takasago International Corporation, 1-5-1 Nishiyawata, Hiratsuka, Kanagawa, 254-0073, Japan

Four isomers of the L-aspartyl-D-alanine fenchyl esters were prepared as potential peptide sweeteners. L-Aspartyl-D-alanine (+)-α-fenchyl ester and L-aspartyl-D-alanine (−)-*β*-fenchyl ester showed sweetness with potencies 250 and 160 times higher than that of sucrose, respectively. In contrast, L-aspartyl-D-alanine (+)-*β*-fenchyl ester and L-aspartyl-D-alanine (−)-α-fenchyl ester had the highest sweetness potencies at 5700 and 1100 times that of sucrose, respectively. In particular, L-aspartyl-D-alanine (−)-α-fenchyl ester had an excellent sweetness quality; but L-aspartyl-D-alanine (+)-*β*-fenchyl ester did not have an excellent quality of sweetness because it displayed an aftertaste caused by the strong sweetness.

Keywords: *Sweetener; fenchol; L-aspartyl-D-alanine fenchyl esters; aspartic acid derivatives*

INTRODUCTION

The correlation between sweetness and chemical structure of a sweetener was proposed by Schellenberger as the AH-B site theory which is explained by a proton donor and a proton acceptor (*1*, *2* and recent references therein), and by Kier as the dispersion X site theory which is considered a hydrophobic site (*3*) (Figure 1).

Since aspartame (**1**) having AH-B and X sites was found in 1969 (*4*, *5*), many L-aspartyl dipeptides have been synthesized (*6*−*8*). In particular, the sweetness of the L-aspartyl-D,L-aminomalonic methyl fenchyl diester (**2**), which was found by Fujino and co-workers (*9*), is 30000 times more potent than sucrose. In 1980, Yin-Zeng et al. synthesized the four fenchyl alcohol isomers of (**2**) and found that the (+)-*β*-fenchyl ester was 50000 times as sweet as sucrose (*10*). This (+)-*β*-fenchyl ester of (**2**) is known as the most potent among these L-aspartyl dipeptides. However, the compound is extremely unstable for practical use, and also the stability of aspartame in aqueous solution is not sufficient because it has a methyl ester unit. To develop good quality, potent sweetness, and improved chemical stability of the L-aspartyl dipeptides, we converted the ester unit into an amino alcohol. As a result, we have recently reported the new sweetener, L-aspartyl fenchyl amino alcohol (**3**) (*11*, *12*), which has a high degree of sweetness and good stability in solution. For the sweetness of the L-aspartyl dipeptides, compounds having a fenchyl unit as a large hydrophobic group (*13*, *14*) have a potent sweetness. We also described L-aspartyl-D-alanine fenchyl esters (**4**) in a previous patent (*15*, *16*). Similar compounds have been reported by Zeng et al. (*17*); however, their reported data left us doubtful about the evaluated sweetness potency.

These authors reported that the L-aspartyl-D-alanine (+)-α-fenchyl ester (**4a**) had no sweetness. Therefore, we independently synthesized four isomers of the L-aspartyl-D-alanine fenchyl esters (**4a**−**d**) (Table 1) to reinvestigate the correlation between the fenchyl isomers and the sweetness potency.

* To whom correspondence should be addressed. Tel: 81-(0)463-21-7150. Fax: 81-(0)463-23-1655. E-mail: TIC00488@nifty.ne.jp.

MATERIALS AND METHODS

Taste Panels. The compounds were dissolved in water, and the threshold value of each compound was determined by a limiting method using a panel consisting of five skilled flavorists; and then these sweetness characteristics were evaluated by 10 panelists.

Instrumentation. Following are the instruments and specifications used for the analyses. IR: Jasco IR-810. ^{1}H NMR (TMS as internal standard): Bruker AM-400 (400 MHz). ^{13}C NMR (TMS as internal standard): Bruker AM-400 (100 MHz). MS: Hitachi M-80A mass spectrometer at 70 eV. Optical rotations: Jasco DIP-4 digital polarimeter. GLC: HP5890 II with an FID detector; column NB-1 (25 m × 0.25 mm i.d., df = 0.15 *μ*m); temperature programmed at 100−200 °C, +1.0 °C/min; carrier gas He, 0.1 MPa. HPLC: Hitachi L-6000 with an L-4000 UV as a detector; column, Inertsil ODS-2 (250 mm × 4.6 mm); eluent, CH_3CN/H_2O, 7:3 (pH 2.3; adjusted by using H_3PO_4); flow rate 0.5 mL/min; detection, UV (210 or 254 nm). Column chromatography: Merck Kieselgel 60, Art. Nr. 7734. Melting points: Yanagimoto micromelting apparatus, uncorrected values.

Preparation of Fenchols. Commercial (+)-α-fenchol (10 g, Aldrich, 95.9% purity) was dissolved in 20 mL of *n*-heptane, and the solution was cooled to −40 °C. The crystals that precipitated were separated by filtration to give 3.9 g of 98.3% purity (+)-α-fenchol. (α/*β* = 98.3/1.6), mp. 40−42 °C. [Lit. 42−43 °C (*18*)], $[\alpha]_D^{26} = +11.0°$ (*c* = 5, in EtOH). [Lit. $[\alpha]_D = +10.5°$ (*c* = 5, in EtOH) (*18*)].

(−)-α-Fenchol was prepared by the lithium aluminum hydride reduction (THF, 0 °C, 16 h) of (+)-fenchone (20 g, Tokyo Kasei, 98% purity), and the crude crystals obtained (17.6 g) were recrystallized from 35 mL of *n*-heptane at −40 °C to afford 3.9 g of 98.1% purity (−)-α-fenchol (α/*β* = 98.1/1.9), mp. 42−44.5 °C, [Lit. 43.6−44.5 °C (*19*)], $[\alpha]_D^{26} = -12.8°$ (*c* = 5, in EtOH), [Lit. $[\alpha]_D^{27} = -12.7°$ (*c* = 3, in 95% EtOH) (*19*)].

(+)-*β*-Fenchol was prepared by the aluminum isopropoxide reduction of (−)-fenchone (25 g, Aldrich, 98% purity, in 2-propanol, reflux, 420 h), and the crude product (14 g, GC analysis showed a 22/78 ratio of (+)-α-fenchol/(+)-*β*-fenchol) was converted to the *p*-nitrobenzoate, recrystallized from *n*-heptane at −40 °C, and followed by hydrolysis with NaOH to give 1.02 g of 98.8% purity (+)-*β*-fenchol (α/*β* = 1.2/98.8). $[\alpha]_D^{26} = +23.3°$ (*c* = 5, in EtOH), [Lit. $[\alpha]_D = +21.5°$ (*c* = 5, in EtOH) (*20*)]. (−)-*β*-Fenchol was prepared by the procedure outlined above; (+)-fenchone was converted to crude product (GC analysis showed a 25/75 ratio of (−)-α-fenchol/(−)-*β*-

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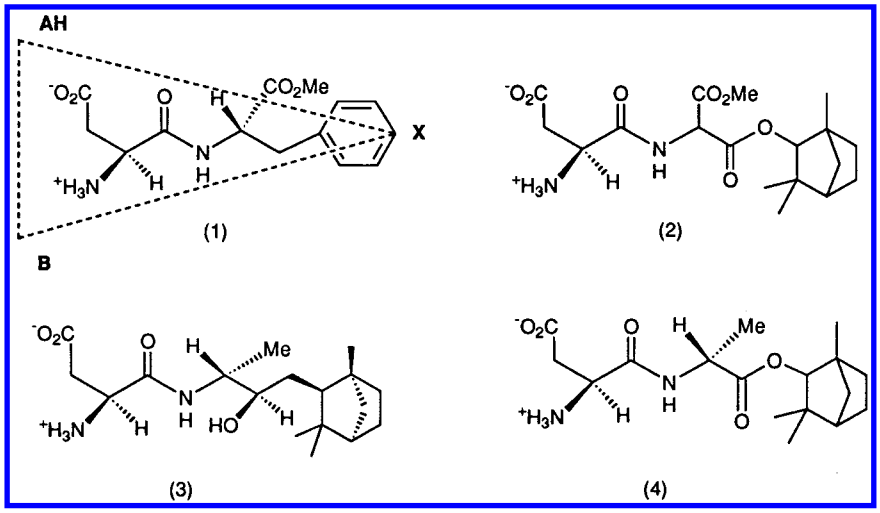
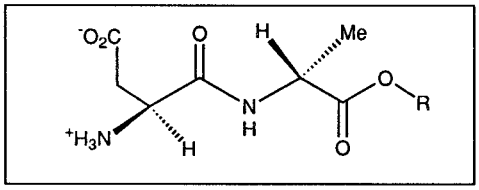
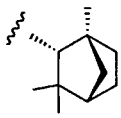
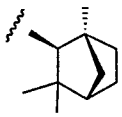
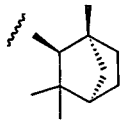
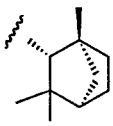


Figure 1. Structures of aspartame (**1**) and L-aspartylfenchyl derivatives (**2**−**4**).

Table 1. Sweetness Characteristics of L-Aspartyl-D-Alanine Fenchyl Esters



Compounds	R		Sweetness[a] potency	Evaluation[c] of quality
(4a)		(+) - α	250 [0][b]	B
(4b)		(+) - β	5,700 [2,600][b]	C
(4c)		(-) - α	1,100 [600][b]	A
(4d)		(-) - β	160 [180][b]	B

[a] Sweetness potency vs 5% sucrose. [b] Value reported by Zeng et al. (*10*). [c] A, Excellent; B, good; C, poor.

fenchol). The crude fenchol was purified by recrystallization after conversion to the *p*-nitrobenzoate and then hydrolyzed with NaOH to give 1.52 g of 99.3% purity (−)-*β*-fenchol (α/*β* = 0.7/99.3). $[\alpha]_D^{26} = -31.60°$ (*c* = 5, in EtOH), [Lit. $[\alpha]_D = -21.8°$ (*c* = 4, in EtOH, 94.4% pure) (*18*)].

Synthesis of *N*-Benzyloxycarbonyl-D-alanine Fenchyl Esters (5a−d). A 2.23-g (10 mM) aliquot of *N*-benzyloxycarbonyl-D-alanine and 1.7 g (11 mM) of one of the fenchol isomers were dissolved in dichloromethane (25 mL) and cooled to 0 °C. Dicyclohexylcarbodiimide [2.49 g (12 mM)] and 14 mg (0.1 mM) of 4-(dimethylamino)pyridine were added, and the mixture was stirred for 30 min, then warmed to room temperature for 6 h. The dicyclohexylurea that formed was filtered off, and the filtrate was washed with 10% citric acid solution, 5% sodium bicarbonate solution, and brine, then dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure. The residue was purified by silica gel chromatography using chloroform as the eluent to give one of the following *N*-benzyloxycarbonyl-D-alanine fenchyl esters as a solid.

***N*-Benzyloxycarbonyl-D-alanine (+)-α-Fenchyl Ester (5a).** Yield 72.5%. Purity by HPLC was 98.5%. mp 90−92 °C. $[\alpha]_D^{26} = +26.0°$ (*c* = 2.0, in $CHCl_3$). IR ($CHCl_3$): *ν* 3430 cm^{-1}, 1720, 1510. ^1H NMR ($CDCl_3$): *δ* 0.77 (3H, s, CH_3), 1.04 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.09−1.74 (7H, m, fenchyl), 1.45 (3H, d, *J* = 7.1 Hz, CH_3CHNH), 4.39 (1H, d, *J* = 1.8 Hz), 4.44

(1H, q, J = 7.1 Hz, CH_3CHNH), 5.11 (2H, s, $PhCH_2$), 7.29−7.36 (5H, m, aromH). ^{13}C NMR ($CDCl_3$): δ 19.0 (q), 19.3 (q), 20.2 (q), 25.8 (t), 26.5 (t), 29.6 (q), 39.5 (s), 41.3 (t), 48.3 (d), 48.4 (s), 49.8 (d), 66.9 (t), 87.4 (d), 128.1 (2×d), 128.2 (d), 128.5 (2×d), 136.4 (s), 155.6 (s), 173.3 (s). MS m/z (%): 359 (2) (M^+), 223 (17), 206 (7), 178 (26), 153 (20), 137 (34), 107 (36), 91 (100). Anal. $C_{21}H_{29}NO_4$ (359.5): calcd, C 70.16, H 8.13, N 3.89; found, C 70.19, H 8.15, N 3.91%.

***N*-Benzyloxycarbonyl-D-alanine (+)-*β*-Fenchyl Ester (5b).** Yield 65%. Purity by HPLC was 98.8%. mp 70−73 °C. $[\alpha]_D^{23} = -18.7°$ (c = 1.5, in $CHCl_3$). IR ($CHCl_3$): ν 3430 cm^{-1}, 1720, 1510. ^1H NMR ($CDCl_3$): δ 0.85 (3H, s, CH_3), 1.01 (3H, s, CH_3), 1.06 (3H, s, CH_3), 1.08−1.75 (7H, m, fenchyl), 1.44 (3H, d, J = 7.1 Hz, CH_3CHNH), 4.19 (1H, d, J = 1.3 Hz), 4.40 (1H, q, J = 7.1 Hz, CH_3CHNH), 5.11 (2H, s, $PhCH_2$), 5.35 (1H, br d, J = 6.9 Hz, NH), 7.29−7.36 (5H, m, aromH). ^{13}C NMR ($CDCl_3$): δ 17.0 (q), 18.9 (q), 23.4 (q), 25.4 (t), 25.6 (q), 33.3 (t), 41.7 (t), 43.7 (s), 48.3 (d), 48.4 (d), 49.8 (d), 66.8 (t), 88.6 (s), 128.1 (2×d), 128.2 (d), 128.5 (2×d), 136.3 (s), 155.6 (s), 172.9 (s). MS m/z (%): 360 (2) (M^++1), 270 (8), 224 (50), 206 (6), 178 (26), 153 (22), 137 (91), 107 (32), 91 (100). Anal. $C_{21}H_{29}NO_4$ (359.5): calcd, C 70.16, H 8.13, N 3.89; found, C 70.18, H 8.16, N 3.90%.

***N*-Benzyloxycarbonyl-D-alanine (−)-α-Fenchyl Ester (5c).** Yield 71%. Purity by HPLC was 99%. mp 70−72 °C. $[\alpha]_D^{26} = -26.0°$ (c = 2.0, in $CHCl_3$). IR ($CHCl_3$): ν 3430 cm^{-1}, 1720, 1510. ^1H NMR ($CDCl_3$): δ 0.78 (3H, s, CH_3), 1.03 (3H, s, CH_3), 1.09 (3H, s, CH_3), 1.07−1.74 (7H, m, fenchyl), 1.44 (3H, d, J = 7.1 Hz, CH_3CHNH), 4.39 (1H, d, J = 1.5 Hz), 4.41 (1H, q, J = 7.1 Hz, CH_3CHNH), 5.11 (2H, s, $PhCH_2$), 7.29−7.37 (5H, m, aromH). ^{13}C NMR ($CDCl_3$): δ 19.1 (q), 19.3 (q), 20.1 (q), 25.8 (t), 26.6 (t), 29.6 (q), 39.41 (s), 41.3 (t), 48.3 (d), 48.4 (s), 49.8 (d), 66.9 (t), 87.4 (d), 128.1 (d), 128.2 (d), 128.4 (d), 128.5 (d), 128.6 (d), 136.3 (s), 155.6 (s), 173.3 (s). MS m/z (%): 359 (6) (M^+), 292 (6), 279 (27), 153 (3), 137 (14), 108 (18), 91 (100). Anal. $C_{21}H_{29}NO_4$ (359.5): calcd, C 70.16, H 8.13, N 3.89; found, C 70.22, H 8.14, N 3.92%.

***N*-Benzyloxycarbonyl-D-alanine (−)-*β*-Fenchyl Ester (5d).** Yield 69%. Purity by HPLC was 99.5%. mp 69−71 °C. $[\alpha]_D^{26} = +15.8°$ (c = 2.1, in $CHCl_3$). IR ($CHCl_3$): ν 3430 cm^{-1}, 1720, 1510. ^1H NMR ($CDCl_3$): δ 0.84 (3H, s, CH_3), 1.01 (3H, s, CH_3), 1.06 (3H, s, CH_3), 1.07−1.74 (7H, m, fenchyl), 1.44 (3H, d, J = 7.2 Hz, CH_3CHNH), 4.22 (1H, d, J = 1.6 Hz), 4.41 (1H, q, J = 7.2 Hz, CH_3CHNH), 5.11 (2H, s, $PhCH_2$), 5.35 (1H, br d, J = 6.9 Hz, NH), 7.26−7.36 (5H, m, aromH). ^{13}C NMR ($CDCl_3$): δ 17.0 (q), 18.9 (q), 23.6 (q), 25.5 (t), 25.7 (q), 33.2 (t), 41.7 (t), 43.7 (s), 48.3 (d), 48.4 (d), 49.8 (d), 66.8 (t), 88.5 (s), 128.1 (2×d), 128.2 (d), 128.5 (2×d), 136.4 (s), 155.6 (s), 172.7 (s). MS m/z (%): 359 (6) (M^+), 292 (6), 279 (27), 153 (3), 137 (14), 108 (18), 91 (100). Anal. $C_{21}H_{29}NO_4$ (359.5): calcd, C 70.16, H 8.13, N 3.89; found, C 70.17, H 8.13, N 3.90%.

Synthesis of *N*-Benzyloxycarbonyl-*β*-benzyl-L-aspartyl-D-alanine Fenchyl Esters (6a−d). A 1.3-g (3.6 mM) aliquot of each *N*-benzyloxycarbonyl-D-alanine fenchyl ester was dissolved in methanol (25 mL), and 0.4 g of 5% palladium/carbon was added to the solution and followed by hydrogenation at room temperature for 5 h under atmospheric pressure. After the catalyst was filtered off, the solvent was removed under reduced pressure to give the D-alanine fenchyl ester as an oil.

A 1.3-g (3.6 mM) aliquot of *N*-benzyloxycarbonyl-L-aspartic acid *β*-benzyl ester was dissolved in dry dioxane (20 mL), and 0.74 g (4 mM) of *N*-hydroxy-*endo*-5-norbornene-2,3-dicarboximide was added to the solution. After the mixture was cooled on ice, 0.84 g (4.1 mM) of dicyclohexylcarbodiimide was added; the temperature was then raised to room temperature and stirring was continued for 4 h. The dicyclohexyl urea that formed was then filtered off, and the previously prepared D-alanine fenchyl ester in dry dioxane (10 mL) was added to the filtrate with ice cooling. The mixture was stirred at room temperature for 16 h, and the solvent was removed under reduced pressure. To the residue, ethyl acetate (60 mL) was added and washed with 10% citric acid solution, 5% sodium bicarbonate solution, and brine. The organic layer was dried with anhydrous magnesium sulfate and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography using chloroform as the eluent to give one of the following N-benzyloxycarbonyl-*β*-benzyl-L-aspartyl-D-alanine fenchyl esters as an oil.

***N*-Benzyloxycarbonyl-*β*-benzyl-L-aspartyl-D-alanine (+)-α-Fenchyl Ester (6a).** Yield 79%. Purity by HPLC was 99.5%. $[\alpha]_D^{24} = +17.6°$ (c = 0.6, in $CHCl_3$). IR ($CHCl_3$): ν 3410 cm^{-1}, 1725, 1675, 1500. ^1H NMR ($CDCl_3$): δ 0.77 (3H, s, CH_3), 1.04 (3H, s, CH_3), 1.11 (3H, s, CH_3), 1.10−1.77 (7H, m, fenchyl), 1.39 (3H, d, J = 7.1 Hz, CH_3CHNH), 2.75 (1H, dd, J = 6.2, 17 Hz, CH_2CO_2Bn), 3.06 (1H, dd, J = 3.8, 17 Hz, CH_2CO_2Bn), 4.39 (1H, d, J = 1.9 Hz, CO_2CH−), 4.57 (1H, q, J = 7.1 Hz, CH_3CHNH). 4.64 (1H, br s, $CHCH_2CO_2Bn$), 5.08−5.17 (4H, m, 2×$PhCH_2$), 7.26−7.37 (10H, m, aromH). ^{13}C NMR ($CDCl_3$): δ 18.5 (q), 19.3 (q), 20.3 (q), 25.8 (t), 26.5 (t), 29.6 (q), 36.1 (t), 39.5 (s), 41.3 (t), 48.3 (d), 48.4 (s), 48.5 (d), 51.2 (d), 66.9 (t), 67.4 (t), 87.5 (d), 128.1 (d), 128.2 (2×d), 128.3 (2×d), 128.4 (2×d), 128.6 (3×d), 135.4 (s), 135.9 (s), 169.6 (s), 171.4 (s), 172.7 (s), 180.3 (s). MS m/z (%): 565 (19) (M+1), 429 (24), 411 (9), 383 (9), 321 (58), 275 (14), 222 (16), 181 (23), 137 (74), 91 (100).

***N*-Benzyloxycarbonyl-*β*-benzyl-L-aspartyl-D-alanine (+)-*β*-Fenchyl Ester (6b).** Yield 78%. Purity by HPLC was 99.5%. $[\alpha]_D^{22} = -11.0°$ (c = 1.0, in $CHCl_3$). IR ($CHCl_3$): ν 3410 cm^{-1}, 1725, 1675, 1500. ^1H NMR ($CDCl_3$): δ 0.84 (3H, s, CH_3), 1.00 (3H, s, CH_3), 1.06 (3H, s, CH_3), 1.0−1.76 (7H, m, fenchyl), 1.38 (3H, d, J = 7.2 Hz, CH_3CHNH), 2.75 (1H, dd, J = 6.3, 17 Hz, CH_2CO_2Bn), 3.06 (1H, dd, J = 3.8, 17 Hz, CH_2CO_2Bn), 4.21 (1H, d, J = 1.9 Hz, CO_2CH−), 4.54 (1H, q, J = 7.2 Hz, CH_3CHNH). 4.63 (1H, br s, $CHCH_2CO_2Bn$), 5.08−5.17 (4H, m, 2×$PhCH_2$), 7.26−7.37 (10H, m, aromH). ^{13}C NMR ($CDCl_3$): δ 17.1 (q), 18.5 (q), 23.5 (q), 25.5 (t), 25.7 (q), 33.3 (t), 36.2 (t), 41.7(t), 41.3 (t), 48.3 (d), 48.4 (s), 48.5 (d), 51.1 (d), 66.9 (t), 67.4 (t), 88.7 (d), 128.1(d), 128.2 (2×d), 128.3 (2×d), 128.4 (2×d), 128.5 (d), 128.6 (2×d), 135.4 (s), 135.9 (s), 169.5 (s), 171.4 (s), 172.2 (s), 180.2 (s). MS m/z (%): 566 (5) (M^++2), 519 (4), 429 (27), 411 (22), 383 (12), 312 (18), 268 (50), 222 (16), 181 (40), 137 (72), 91 (100).

***N*-Benzyloxycarbonyl-*β*-benzyl-L-aspartyl-D-alanine (−)-α-Fenchyl Ester (6c).** Yield 81%. Purity by HPLC was 99%. $[\alpha]_D^{23} = -11.9°$ (c = 1.0, in $CHCl_3$). IR ($CHCl_3$): ν 3410 cm^{-1}, 1720, 1675, 1500. ^1H NMR ($CDCl_3$): δ 0.77 (3H, s, CH_3), 1.03 (3H, s, CH_3), 1.09 (3H, s, CH_3), 1.07−1.74 (7H, m, fenchyl), 1.38 (3H, d, J = 7.1 Hz, CH_3CHNH), 2.75 (1H, dd, J = 6.4, 17 Hz, CH_2CO_2Bn), 3.07 (1H, dd, J = 3.8, 17 Hz, CH_2CO_2Bn), 4.04 (1H, d, J = 1.9 Hz, CO_2CH−), 4.56 (1H, q, J = 7.1 Hz, CH_3CHNH). 4.63 (1H, br s, $CHCH_2CO_2Bn$), 5.08−5.17 (4H, m, 2×$PhCH_2$), 7.26−7.37 (10H, m, aromH). ^{13}C NMR ($CDCl_3$): δ 18.6 (q), 19.3 (q), 20.1 (q), 25.8 (t), 26.7 (t), 29.7 (q), 36.2 (t), 39.4 (s), 41.3 (t), 48.3 (d), 48.4 (s), 48.6 (d), 51.1 (d), 66.9 (t), 67.4 (t), 87.4 (d), 128.1 (d), 128.2 (2×d), 128.3 (2×d), 128.4 (2×d), 128.6 (3×d), 135.4 (s), 135.9 (s), 169.5 (s), 171.4 (s), 172.7 (s), 180.3 (s). MS m/z (%): 565 (9) (M^++1), 429 (4), 411 (9), 383 (5), 321 (12), 275 (30), 222 (17), 181 (23), 137 (72), 91 (100).

N−Benzyloxycarbonyl-*β*-benzyl-L-aspartyl-D-alanine (−)-*β*-Fenchyl Ester (6d). Yield 77%. Purity by HPLC was 99.5%. $[\alpha]_D^{23} = +18.4°$ (c = 0.5, in $CHCl_3$). IR ($CHCl_3$): ν 3410 cm^{-1}, 1725, 1680, 1500. ^1H NMR ($CDCl_3$): δ 0.84 (3H, s, CH_3), 1.00 (3H, s, CH_3), 1.07 (3H, s, CH_3), 1.09−1.76 (7H, m, fenchyl), 1.39 (3H, d, J = 7.3 Hz, CH_3CHNH), 2.75 (1H, dd, J = 6.2, 17.1 Hz, CH_2CO_2Bn), 3.04 (1H, dd, J = 4.1, 17.1 Hz, CH_2CO_2Bn), 4.21 (1H, d, J = 1.9 Hz, CO_2CH−), 4.55 (1H, q, J = 7.3 Hz, CH_3CHNH). 4.62 (1H, br s, $CHCH_2CO_2Bn$), 5.08−5.17 (4H, m, 2×$PhCH_2$), 7.26−7.37 (10H, m, aromH). ^{13}C NMR ($CDCl_3$): δ 17.1 (q), 18.4 (q), 23.6 (q), 25.6 (t), 25.7 (q), 33.3 (t), 36.1 (t), 41.7 (t), 43.8 (t), 48.3 (s), 48.4 (d), 48.5 (d), 51.1 (d), 66.9 (t), 67.4 (t), 88.7 (d), 128.1(d), 128.2 (2×d), 128.3 (2×d), 128.4 (2×d), 128.6 (3×d), 135.4 (s), 135.9 (s), 169.5 (s), 171.4 (s), 172.7 (s), 180.3 (s). MS m/z (%): 565 (5) (M^++1), 429 (33), 411 (10), 385 (14), 321 (29), 268 (17), 222 (19), 181 (21), 137 (70), 91 (100).

Synthesis of L-aspartyl-D-alanine Fenchyl Esters (4a−d). 1.1 g (1.95 mM) of *N*-benzyloxycarbonyl-*β*-benzyl-L-aspartyl-D-alanine fenchyl ester was dissolved in methanol (20 mL),

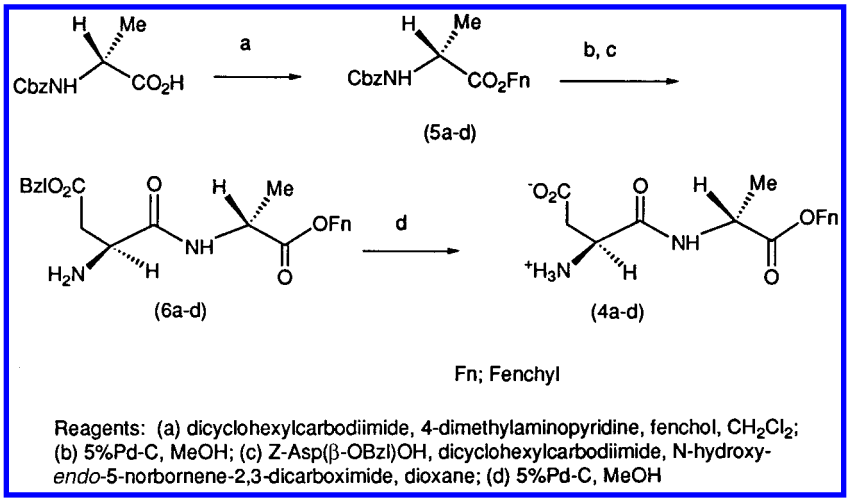


Figure 2. Synthesis of **4a**−**d**.

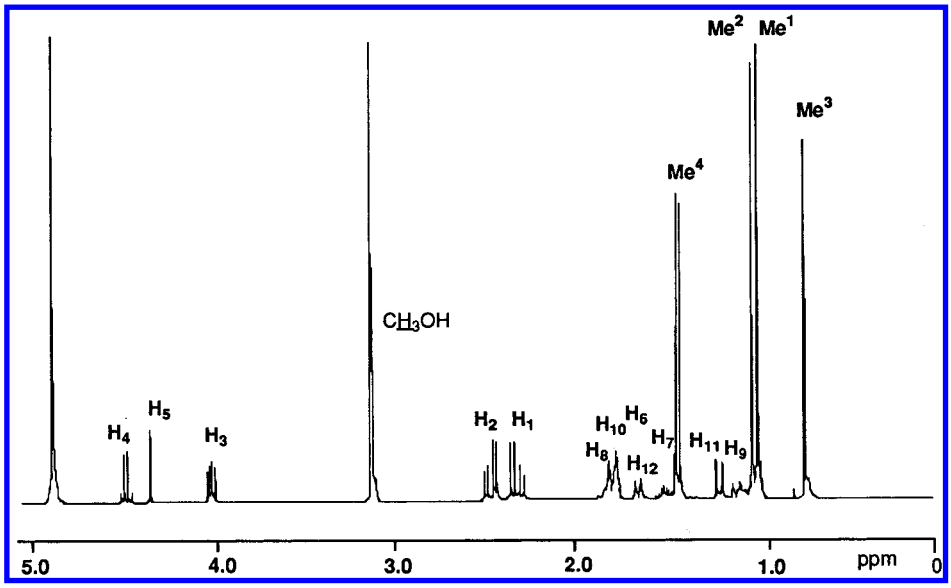


Figure 3. ^{1}H NMR spectrum of L-aspartyl-D-alanine (−)-α-fenchyl ester.

and 0.3 g of 5% palladium/carbon was added to the solution followed by hydrogenation at room temperature for 5 h under atmospheric pressure. After the catalyst was filtered off, the solvent was removed under reduced pressure. *n*-Hexane was added to the residue, forming a powdery precipitate. The precipitate was separated by filtration and dried to give one of following the L-aspartyl-D-alanine fenchyl esters as a colorless and odorless powder.

L-Aspartyl-D-alanine (+)-α-Fenchyl Ester (4a). Yield 83%. Purity by HPLC was 100%. mp 138−141 °C. $[\alpha]_D^{25}$ = +51.6° (*c* = 0.55, in MeOH). IR ($CHCl_3$): *ν* 3400 cm^{-1}, 3220, 1735, 1685. ^{1}H NMR (CD_3OD): *δ* 0.81 (3H, s, CH_3), 1.06 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.04−1.84 (7H, m, fenchyl), 1.45 (3H, d, *J* = 7.3 Hz, C*H*$_3$CHNH), 2.57 (1H, dd, *J* = 9.2, 17 Hz, C*H*$_2$CO$_2$H), 2.71 (1H, dd, *J* = 4.7, 17 Hz, C*H*$_2$CO$_2$H), 4.06 (1H, dd, *J* = 4.7, 9.2 Hz, C*H*CH$_2$CO$_2$H), 4.38 (1H, d, *J* = 1.9 Hz, CO$_2$C*H*−), 4.49 (1H, q, *J* = 7.3 Hz, CH$_3$C*H*NH). ^{13}C NMR (CD_3-OD): *δ* 17.8 (q), 19.6 (q), 20.9 (q), 26.8 (t), 27.5 (t), 30.1 (q), 34.6 (s), 38.3 (t), 40.6 (s), 42.2 (t), 48.6 (d), 49.3 (d), 52.4 (d), 88.6 (d), 170.1 (s), 174.4 (s), 176.0 (s). MS *m*/*z* (%): 341 (2) (M^++1), 187 (26), 169 (5), 159 (40), 141 (10), 137 (13), 113 (4), 102 (19), 88 (100), 81 (12), 70 (4), 44 (38). Anal $C_{17}H_{26}N_2O_5$ (340.4): calcd, C 59.98, H 8.29, N, 8.23; found, C 60.12, H 8.40, N 8.09%.

L-Aspartyl-D-alanine (+)-*β*-Fenchyl Ester (4b). Yield 80%. Purity by HPLC was 100%. mp 114−117 °C. $[\alpha]^{25}{}_D$ = +10.9° (*c* = 0.55, in MeOH). IR ($CHCl_3$): *ν* 3325 cm^{-1}, 3200, 1730, 1690. ^{1}H NMR (CD_3OD): *δ* 0.89 (3H, s, CH_3), 1.04 (3H, s, CH_3), 1.05 (3H, s, CH_3), 1.13−1.82 (7H, m, fenchyl), 1.44 (3H, d, *J* = 7.3 Hz, C*H*$_3$CHNH), 2.83 (1H, dd, *J* = 8.5, 17.7 Hz, C*H*$_2$CO$_2$H), 2.94 (1H, dd, *J* = 4.6, 17.7 Hz, C*H*$_2$CO$_2$H), 4.18 (1H, q, *J* = 4.6, 8.5 Hz, C*H*CH$_2$CO$_2$H), 4.17 (1H, d, *J* = 1.8 Hz, CO$_2$C*H*−), 4.47 (1H, q, *J* = 7.3 Hz, CH$_3$C*H*NH). ^{13}C NMR (CD_3OD): *δ* 17.4 (q), 17.5 (q), 24.0 (q), 26.1 (t), 26.4 (t), 32.7 (s), 34.4 (t), 36.6 (t), 42.6 (t), 44.9 (s), 48.8 (d), 49.9 (d), 51.3 (d), 90.0 (d), 169.1 (s), 173.6 (s), 174.0 (s). MS *m*/*z* (%): 341 (4) (M^++1), 187 (25), 169 (4), 159 (38), 141 (8), 137 (15), 113 (4), 102 (8), 88 (100), 81 (15), 70 (4), 44 (31). Anal $C_{17}H_{26}N_2O_5$ (340.4): calcd, C 59.98, H 8.29, N 8.23; found, C 60.07, H 8.31, N 8.19%.

L-Aspartyl-D-alanine (−)-α-Fenchyl Ester (4c). Purity by HPLC was 100%. Yield 80%. mp 128−133 °C. $[\alpha]_D^{25}$ = +9.6° (*c* = 0.35, in MeOH). IR ($CHCl_3$): *ν* 3350 cm^{-1}, 3200, 1735, 1685. ^{1}H NMR (CD_3OD): *δ* 0.82 (3H, s, CH_3), 1.05 (3H, s, CH_3), 1.08 (3H, s, CH_3), 1.03−1.83 (7H, m, fenchyl), 1.44 (3H, d, *J* = 7.3 Hz, C*H*$_3$CHNH), 2.61 (1H, dd, *J* = 9.1, 17 Hz, C*H*$_2$CO$_2$H), 2.74 (1H, dd, *J* = 4.8, 17 Hz, C*H*$_2$CO$_2$H), 4.08 (1H, dd, *J* = 4.8, 9.1 Hz, C*H*CH$_2$CO$_2$H), 4.38 (1H, d, *J* = 2 Hz, CO$_2$C*H*−), 4.49 (1H, q, *J* = 7.3 Hz, CH$_3$C*H*NH). ^{13}C NMR (CD_3OD): *δ* 17.7 (q), 19.6 (q), 20.6 (q), 26.7 (t), 27.5 (t), 30.0 (q), 34.4 (s), 38.0 (t), 40.5 (s), 42.2 (t), 48.9 (d), 49.3 (d), 52.2 (d), 88.6 (d), 170.1 (s), 174.5 (s), 175. 7(s). MS *m*/*z* (%): 341 (2) (M^++1), 187 (27), 169 (8), 159 (42), 141 (13), 137 (13), 113 (6), 102 (27), 88 (100), 81 (12), 70 (4). Anal $C_{17}H_{26}N_2O_5$ (340.4): calcd, C 59.98, H 8.29, N 8.23; found, C 60.22, H 8.42, N 8.03%.

L-Aspartyl-D-alanine (−)-*β*-Fenchyl Ester (4d). Yield 79%. Purtity by HPLC was 100%. mp 131−136 °C. $[\alpha]_D^{25}$ = +33.3° (*c* = 0.33, in MeOH). IR ($CHCl_3$): *ν* 3350 cm^{-1}, 3200,

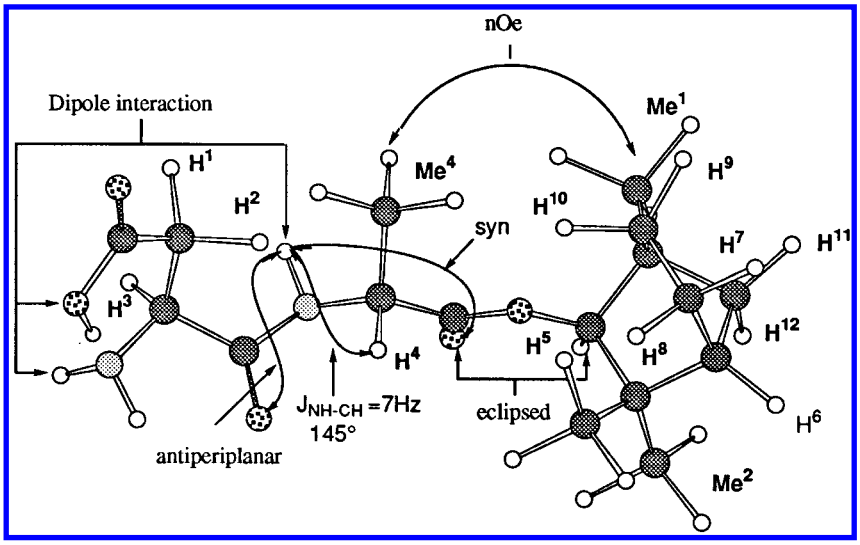


Figure 4. The estimated conformation of L-aspartyl-D-alanine (−)-α-fenchyl ester.

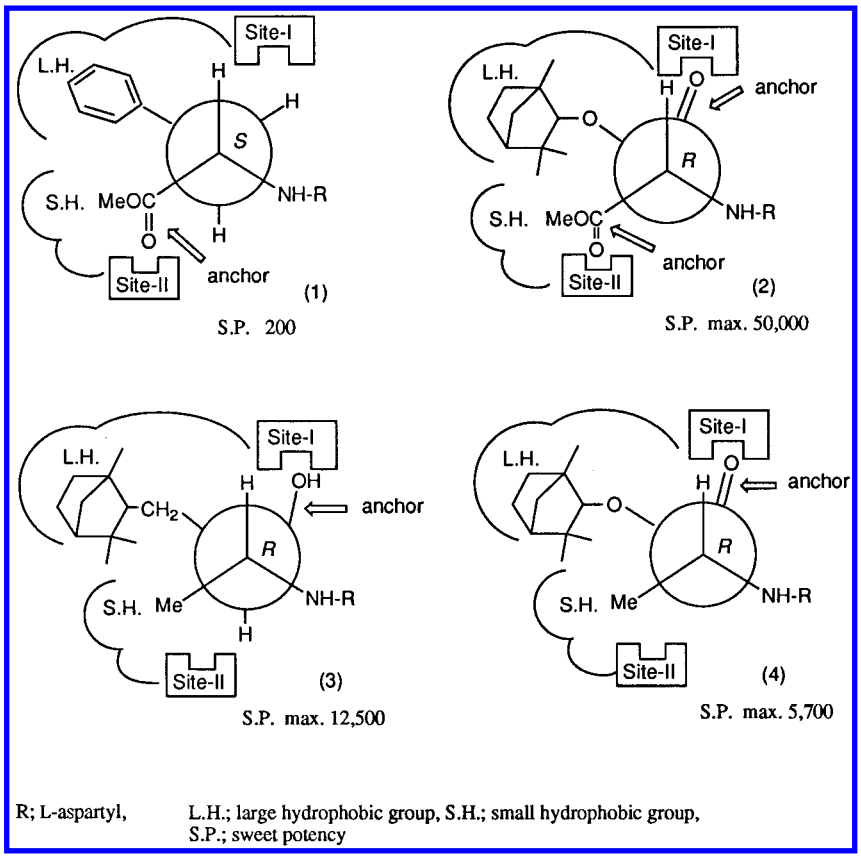


Figure 5. Newman projections of sweeteners **1**−**4**.

1730, 1690. ^{1}H NMR (CD_3OD): δ 0.89 (3H, s, CH_3), 1.04 (3H, s, CH_3), 1.07 (3H, s, CH_3), 1.13−1.82 (7H, m, fenchyl), 1.44 (3H, d, J = 7.3 Hz, CH_3CHNH), 2.85 (1H, dd, J = 8.7, 17.8 Hz, CH_2CO$_2$H), 2.97 (1H, dd, J = 4.4, 17.9 Hz, CH_2CO$_2$H), 4.18 (1H, dd, J = 4.4, 8.7 Hz, CHCH$_2$CO$_2$H), 4.19 (1H, d, J = 1.6 Hz, CO$_2$CH−), 4.49 (1H, q, J = 7.3 Hz, CH$_3$CHNH). ^{13}C NMR (CD_3OD): δ 17.4 (q), 17.6 (q), 24.2 (q), 26.0 (t), 26.3 (t), 32.7 (s), 34.3 (t), 36.2 (t), 42.6 (t), 44.8 (s), 49.1 (d), 49.2 (d), 51.0 (d), 89.9 (d), 168.9 (s), 172.9 (s), 173.8 (s). MS m/z (%); 341 (4) (M^++1), 187 (23), 169 (7), 159 (39), 141 (10), 137 (15), 113 (5), 102 (27), 88 (100), 81 (14), 70 (3), 44 (38). Anal $C_{17}H_{26}N_2O_5$ (340.4): calcd, C 59.98, H 8.29, N 8.23; found, C 60.02, H 8.38, N 8.19%.

RESULTS AND DISCUSSION

The syntheses of the L-aspartyl-D-alanine fenchyl esters (**4a**−**d**) were accomplished as follows: Condensation of *N*-benzyloxycarbonyl-D-alanine with fenchol by dicyclohexylcarbodiimide and a catalytic amount of 4-(dimethylamino)pyridine gave the corresponding *N*-benzyloxycarbonyl-D-alanine fenchyl esters (**5a**−**d**) in 65−72.5% yield, which was followed by deprotection by hydrogenolysis with 5% palladium/carbon. The fenchyl esters of D-alanine (**4a**−**d**) were condensed with *N*-benzyloxycarbonyl-β-benzyl-L-aspartic acid by dicyclohexylcarbodiimide and a catalytic amount of *N*-hydroxy-*endo*-

5-norbornene-2,3-diimide to give the *N*-benzyloxycarbonyl-*β*-benzyl-L-aspartyl-D-alanine fenchyl esters (**6a**−**d**) in 77−81% yield, which was followed by deprotection by hydrogenolysis with 5% palladium/carbon to give the L-aspartyl-D-alanine fenchyl ester as a colorless powder (Figure 2).

We compared the sweetness potency of the L-aspartyl fenchyl esters (**4a**−**d**) with that of sucrose. As a result of the evaluation, the (+)-α and (−)-*β* isomers (**4a** and **4d**) showed intensities of 250 and 160 times sucrose, respectively. On the other hand, the sweetness of the (+)-*β*- form (**4b**) was 5700 times and the (−)-α- form (**4c**) was 1100 times greater than that of sucrose (Table 1). From this result, the reported data of Zeng et al. that showed **4a** had no sweetness were corrected. We further assumed that the configuration of the (+)-*β*- or (−)-α-fenchyl unit is a better fitting form for the sweetness receptor.

Next, we studied the conformation of L-aspartyl-D-alanine (−)-α-fenchyl ester (**4c**) by NMR (Figure 3). Each proton was assigned by 2D NMR, and the nuclear Overhauser effect (nOe) in L-aspartyl-D-alanine (−)-α-fenchyl ester (**4c**) was observed between the bridgehead methyl group (Me^1) of fenchyl and the methyl group (Me^4) of alanine (Figure 4). The coupling constant of NH and the methine proton (H^4) of the alanine unit ($J_{HN}\alpha$) was 7 Hz by selective frequency decoupling (SFDEC) and thus their dihedral angle was calculated to be 145° (*21*). The carbonyl oxygen atom of the ester and the 2-position proton of the bicyclo ring is considered to be eclipsed. Also, the conformation between the carbonyl of the amide and NH is considered to be antiperiplanar (Figure 4).

The Newman projections of aspartame (**1**), the aminomalonic fenchyl ester (**2**) (*9*, *10*), the fenchyl amino alcohol (**3**) (*11*, *12*), and the D-alanine fenchyl ester (**4**) prepared by us are shown in Figure 5. From these Newman projections, we propose the following hypothesis: Sites I and II are present in the sweet taste receptors for fitting the large and small hydrophobic groups (*22*) of these sweeteners, and these sites have a nonbonding interaction to functional groups; for example, the carbonyl of the ester or hydroxy function. These functional groups act only as an anchor. Sweetener **2** has two anchors to fit both sites I and II, and therefore, has the highest sweetness potency. On the other hand, sweeteners **3**, **4**, and **1** have one anchor to fit site II or I (Figure 5). We assume that site I has a higher sweetness potency than site II.

In particular, **4c** has an excellent quality, potent sweetness, and is more stable than aspartame in solution, but not sufficient for use as a sweetener. **4b** did not have an excellent quality of sweetness because it displayed an aftertaste caused by the strong sweetness. Unfortunately, **4b** and **4c** were found to be slowly hydrolyzed at the pH (3−4) of soft drinks to give fenchol, a compound that imparts an undesirable off-taste to the beverage at levels of less than 0.1 ppm (*23*). We have previously reported that the D-alanine unit can be changed to the more stable structure of the optically active amino alcohol (*11*, *12*).

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