Synthesis of Aspartyl Pentapeptide Esters in Relation to Structural Features of Sweet Peptides

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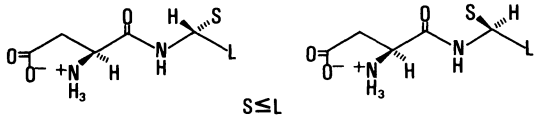
(Received November 13, 1985)

A series of seven analogues of aspartyl pentapeptides has been synthesized in relation to the structural features of sweet peptides. The rule in the structure-taste relationships of di-, tri-, and tetrapeptides is inapplicable to the pentapeptides. All the pentapeptides were devoid of sweetness, though they satisfied the requirements for sweet peptides. The result indicates that oligopeptides can not fit a deep receptor pocket. The mode of interactions between sweet peptides and the receptor is drawn schematically.

In a previous paper,[1] the tastes of aspartyl dipeptide esters have been rationalized through Fischer projection formulas. Recently, in order to deduce the mode of interactions between the sweet peptides and the receptor site, various aspartyl tri- and tetrapeptide esters have been synthesized.[2,3] In the structures (Ia, II, and III), a small alkyl group (S or R^2) has been considered to participate in binding with the receptor through a hydrophobic interaction. Another hydrophobic interaction at R^4 with the receptor has also been suggested.[2] The carboxyl and amino groups serve as a proton acceptor and a proton donor, respectively, in the hydrogen bonding with the receptor. Another hydrogen bonding at the peptide bond with the receptor has also been suggested by the fact that any modification of the peptide bond resulted in a complete loss of sweetness.[4,5] The shape and size of a molecule are important, since the receptor site is considered to be in the shape of a deep pocket[6] or a narrow cleft.[7] Another important factor, common to all sweet compounds, is the hydrophilic-hydrophobic balance in a molecule. In this paper, a series of seven analogues of aspartyl pentapeptides was synthesized in order to obtain further information about the mode of interactions between the sweet peptides and the receptor site.

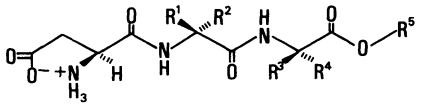


Ia Sweet Ib Nonsweet

Fig. 1. General structure for sweet aspartyl dipeptide esters: S=small hydrophobic group (1—4 atoms); L=larger hydrophobic group (3—6 atoms).[1,2]

Synthesis

Syntheses of Boc-tripeptide esters and the removal of the Boc group were carried out according to the method described in a previous paper.[3] The protected tetrapeptides in Table 1 were prepared by condensation of the appropriate tripeptide ester with a Boc-amino



II

Fig. 2. L-α-Aspartyl tripeptide esters.

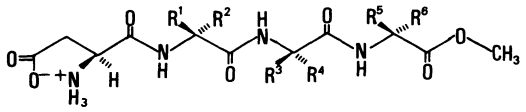


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Fig. 3. L-α-Aspartyl tetrapeptide esters.

Table 1. Protected Tetrapeptides

No.	Compound[a]	Yield %	Mp θ_m/°C	Recrys. solvent[b]	Appearance[c]	$[\alpha]_D^{25}$ Degree[d]	Formula[e]
1	Boc-D-Ala-L-Ala-L-Ala-L-Ala-OMe	59.8	196—197	E	N	−38.7	$C_{18}H_{32}O_7N_4$
2	Boc-D-Ala-L-Ala-L-Val-L-Ala-OMe	79.6	213—214	C	N	−52.9	$C_{20}H_{36}O_7N_4$
3	Boc-D-Ala-L-Val-L-Ala-L-Ala-OMe	59.6	230—231	E	N	−42.2	$C_{20}H_{36}O_7N_4$
4	Boc-D-Ala-L-Val-L-Val-L-Ala-OMe	87.8	219—220	E	N	−47.2	$C_{22}H_{40}O_7N_4$
5	Boc-D-Ala-L-Val-L-Ala-L-Leu-OMe	84.9	214—215	E	N	−40.4	$C_{23}H_{42}O_7N_4$
6	Boc-D-Val-L-Ala-L-Val-L-Ala-OMe	84.4	232—233	E	P	−55.4[f]	$C_{22}H_{40}O_7N_4$
7	Boc-L-Ala-L-Val-L-Val-L-Ala-OMe	79.4	231—232	E	N	−98.5	$C_{22}H_{40}O_7N_4$

a) Abbreviations follow the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature in *Eur. J. Biochem.*, **138**, 9 (1984). b) Recrystallization solvent: C, Chloroform; E, Ethyl acetate. c) Appearance: N, Needles; P, Powder. d) In methanol, c=1.0%. e) All compounds were analyzed for C, H, and N, and the results were within ±0.3% of the theoretical values. Complete analytical data for all compounds have been deposited at the office of the Chemical Society of Japan (Document No. 8622). f) c=0.5%.

Table 2. Protected Pentapeptides, Z-L-Asp(OBzl)-X

Compd No.	X	Yield %	Mp θ_m/°C	Recrys. solvent[a]	$[\alpha]_D^{25}$ Degree[b]	Formula[c]
8	D-Ala-L-Ala-L-Ala-L-Ala-OMe	76.6	222—223	D-W	−27.5	$C_{32}H_{41}O_{10}N_5$
9	D-Ala-L-Ala-L-Val-L-Ala-OMe	80.3	213—215	D-W	−26.9	$C_{34}H_{45}O_{10}N_5$
10	D-Ala-L-Val-L-Ala-L-Ala-OMe	87.1	236—237	E	−27.7	$C_{34}H_{45}O_{10}N_5$
11	D-Ala-L-Val-L-Val-L-Ala-OMe	89.4	239—240	D-W	−24.1	$C_{36}H_{49}O_{10}N_5$
12	D-Ala-L-Val-L-Ala-L-Leu-OMe	67.2	224—225	D-W	−25.8	$C_{37}H_{51}O_{10}N_5$
13	D-Val-L-Ala-L-Val-L-Ala-OMe	80.4	240—241	D-W	−32.5	$C_{36}H_{49}O_{10}N_5$
14	L-Ala-L-Val-L-Val-L-Ala-OMe	85.5	251—252	D-W	−17.1	$C_{36}H_{49}O_{10}N_5$

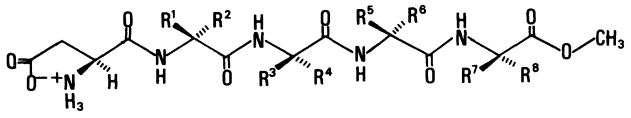
a) Recrystallization solvent: D, *N,N*-Dimethylformamide; W, Water; E, Ethyl acetate. b) In *N,N*-dimethylformamide, c=1.0%. c) All compounds were analyzed for C, H, and N, and the results were within ±0.3% of the theoretical values. Complete analytical data for all compounds have been deposited at the office of the Chemical Society of Japan (Document No. 8622).

Table 3. Pentapeptide Esters, L-α-Asp-X

Compd No.	X	Yield %	R_f[a]	Mp θ_m/°C	Recrys. solvent[b]	Appearance[c]	$[\alpha]_D^{25}$ Degree	Formula[d]
15	D-Ala-L-Ala-L-Ala-L-Ala-OMe	71.9	0.27	202—203 dec	W-A	P	−62.5[e]	$C_{17}H_{29}O_8N_5 \cdot 1.25H_2O$
16	D-Ala-L-Ala-L-Val-L-Ala-OMe	86.5	0.38	219—220	W-A	N	−54.7[e]	$C_{19}H_{33}O_8N_5 \cdot 1.75H_2O$
17	D-Ala-L-Val-L-Ala-L-Ala-OMe	69.6	0.38	205—206 dec	W-A	P	−57.4[e]	$C_{19}H_{33}O_8N_5 \cdot 1.75H_2O$
18	D-Ala-L-Val-L-Val-L-Ala-OMe	64.5	0.48	>245	W	P	−31.6[f]	$C_{21}H_{37}O_8N_5 \cdot 1.5H_2O$
19	D-Ala-L-Val-L-Ala-L-Leu-OMe	61.8	0.49	206—207 dec	W	N	−31.6[f]	$C_{22}H_{39}O_8N_5 \cdot 2H_2O$
20	D-Val-L-Ala-L-Val-L-Ala-OMe	65.2	0.52	>245	W-AA	N	−33.2[g]	$C_{21}H_{37}O_8N_5 \cdot 2.5H_2O$
21	L-Ala-L-Val-L-Val-L-Ala-OMe	40.1	0.44	>245	W	P	−70.6[f]	$C_{21}H_{37}O_8N_5 \cdot 2H_2O$

a) *n*-BuOH:AcOH:H_2O=4:1:1 (v/v). b) Recrystallization solvent: W, Water; A, Acetone; AA, Acetic acid. c) Appearance: P, Powder; N, Needles. d) All compounds were analyzed for C, H, and N, and the results were within ±0.3% of the theoretical values. Complete analytical data for all compounds have been deposited at the office of the Chemical Society of Japan (Document No. 8622). e) In water, c=1.0%. f) In acetic acid, c=0.5%. g) In acetic acid, c=0.25%.

acid *N*-hydroxysuccinimide ester. The Boc group of the tetrapeptide esters was removed with *p*-toluenesulfonic acid (TosOH) in methanol.[8] The protected pentapeptide esters in Table 2 were prepared by condensation of *N*-benzyloxycarbonyl-L-aspartic acid β-benzyl α-succinimide ester (Z-L-Asp(OBzl)-ONSu)[2] with the appropriate tetrapeptide ester. The desired pentapeptide esters in Table 3 were obtained by deprotection of the benzyloxycarbonyl and benzyl groups from the protected pentapeptides in Table 2 by hydrogenation over palladium on charcoal.



IV

Fig. 4. L-α-Aspartyl pentapeptide esters.

Results and Discussion

In order for aspartyl tri- and tetrapeptides to be sweet, the second amino acid must have a D-configuration and a small alkyl side chain.[2,3] Therefore, a small alkyl group was introduced at R^2 so as to meet the sweet structure (II). In structural variation, a pentapeptide (**21**) with an opposite configuration at the chiral center was also synthesized. An L-antipode was introduced for the third amino acid, because the modification of tripeptides (II) showed that an L-configuration at the third amino acid was required for a potent sweet taste.

Most of the pentapeptides were predicted not to be sweet, since aspartyl peptides while elongating their peptide bonds significantly decrease their sweetness potencies and in some cases lose their sweet tastes. As expected, all the pentapeptides synthesized here were essentially tasteless or faintly bitter (Table 4).

The rule in the structure-taste relationships of di-, tri-, and tetrapeptides is inapplicable to pentapeptides. With increasing the length of a peptide, it becomes difficult to fit the narrow receptor pocket. This may be a major reason that some of the tetrapeptides and all of the pentapeptides were not sweet but bitter or tasteless, though they satisfied the requirements for sweet peptides. As a typical case, a series of L-α-aspartyl oligoalanine esters is illustrated in Table 5, in which the sweetness potencies suddenly fall off when the peptide bond is elongated. The taste of aspartyl peptides while elongating their peptide bonds tends to follow Ney's rule[9] for bitter peptides, in which peptides can be classified as bitter or nonbitter, depending upon the magnitude of the hydrophobicity of their side chains.[9] Accordingly, the results obtained for the pentapeptides, in conjunction with the results of di-, tri-, and tetrapeptides, have further supported the previous idea that the receptor site is

Table 4. Taste of Pentapeptides (L-α-Asp-X)[a]

Compd No.	X	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	Taste[b]
15	D-Ala-L-Ala-L-Ala-L-Ala-OMe	H	Me	H	Me	Me	H	H	Me	0
16	D-Ala-L-Ala-L-Val-L-Ala-OMe	H	Me	H	Me	*i*-Pr	H	H	Me	0
17	D-Ala-L-Val-L-Ala-L-Ala-OMe	H	Me	H	*i*-Pr	Me	H	H	Me	—
18	D-Ala-L-Val-L-Val-L-Ala-OMe	H	Me	H	*i*-Pr	*i*-Pr	H	H	Me	0
19	D-Ala-L-Val-L-Ala-L-Leu-OMe	H	Me	H	*i*-Pr	Me	H	H	*i*-Bu	0
20	D-Val-L-Ala-L-Val-L-Ala-OMe	H	*i*-Pr	H	Me	*i*-Pr	H	H	Me	0
21	L-Ala-L-Val-L-Val-L-Ala-OMe	Me	H	H	*i*-Pr	*i*-Pr	H	H	Me	0

a) For structure, see Fig. 4. b) 0=tasteless; —=faintly bitter.

Table 5. Taste of Aspartyl Oligoalanine Esters

Compound	Sweetness value[a]
L-α-Asp-D-Ala-OMe	25[10]
L-α-Asp-D-Ala-OEt	80[10]
L-α-Asp-D-Ala-OPr	125
L-α-Asp-D-Ala-L-Ala-OMe	50
L-α-Asp-D-Ala-L-Ala-L-Ala-OMe	0.5
L-α-Asp-D-Ala-L-Ala-L-Ala-L-Ala-OMe	0

a) Times sucrose (weight basis, sucrose=1).

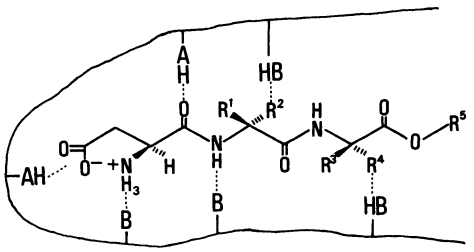


Fig. 5. Schematic drawing of interactions between the sweet peptides and the receptor.
R^1=R^3=H; R^2=R^4=small alkyl group.
AH, Proton donor; B, Proton acceptor; HB, Hydrophobic binding group.

in the shape of a deep pocket with the binding sites inside. The mode of interactions between the sweet peptides and the receptor is illustrated in Fig. 5; here, the case of tripeptides is shown. In the case of Aspartame (L-α-Asp-L-Phe-OMe), the methyl ester group corresponds to R^2 and will interact with the receptor through a hydrophobic interaction, and the phenyl moiety will interact with the receptor at the partner of R^4 through a hydrophobic interaction. These hydrophobic interactions increased the sweetness potency.[1-3] As mentioned above, by increasing the length of a sweet peptide, it becomes difficult to fit the deep receptor. The decrease in its binding ability to the receptor reduces or loses the sweetness potency.

Experimental

All the melting points were taken on a Yanagimoto capillary melting point apparatus Model MP-21 and are uncorrected. Optical rotations were measured on a JASCO DIP-140 digital polarimeter with a 10 cm water-jacketed cell at 25 °C and a 1% concentration. Thin-layer chromatography (TLC) was performed on precoated silica gel $60F_{254}$ plates (E. Merck) and spots were detected with ninhydrin. All compounds were homogeneous on TLC. All of the pentapeptides were almost tasteless or faintly bitter. Therefore, it was judged only whether a peptide was sweet, bitter, or tasteless by tasting crystals of each pentapeptide.

Materials. Boc-amino acids were purchased from Peptide Institute Inc. Esters of L-Ala and L-Leu, and Z-L-Asp(OBzl)-OH were purchased from Kokusan Chemical Works Ltd.

Protected Tetrapeptides (1—7). A typical run (**3** in Table 1) was as follows: To a solution of Boc-L-Val-L-Ala-L-Ala-OMe (2.05 g, 5.5 mmol) in 15 ml of methanol was added TosOH·H_2O (1.26 g, 6.6 mmol). The mixture was stirred at 35 °C for 5 h. The solvent was evaporated under reduced pressure at a bath temperature of 50 °C to give H-L-Val-L-Ala-L-Ala-OMe·TosOH as a semisolid residue. The residue was dissolved in chloroform and the solvent was evaporated under reduced pressure to leave a semisolid residue. The residue was dissolved in 40 ml of chloroform and cooled in an ice-bath. To this solution was added triethylamine (Et_3N, 0.67 g, 6.6 mmol), followed by Boc-D-Ala-ONSu (1.43 g, 5 mmol) with stirring. The mixture was stirred at room temperature for 3 h and then kept standing overnight. The reaction mixture was washed successively with water, a 10% citric acid solution, a 5% sodium hydrogencarbonate solution, and water, and then concentrated under reduced pressure to leave Boc-D-Ala-L-Val-L-Ala-L-Ala-OMe (**3**) as a solid. The solid was crystallized from ethyl acetate–hexane to give **3** as needles. Recrystallization was carried out from ethyl acetate. The data are given in Table 1.

Protected Pentapeptide Esters (8—14). A typical run (**10** in Table 2) was as follows: To a solution of Boc-D-Ala-L-Val-L-Ala-L-Ala-OMe (**3**, 1.10 g, 2.5 mmol) in 20 ml of methanol was added TosOH·H_2O (1.00 g, 5.3 mmol). The mixture was stirred at 40 °C for 4 h. The solvent was evaporated under reduced pressure at a bath temperature of 50 °C to give H-D-Ala-L-Val-L-Ala-L-Ala-OMe·TosOH as an oily residue. The residue was dissolved in chloroform and the solvent was evaporated under reduced pressure. The procedure was repeated twice. The crystalline residue, thus obtained, was suspended in 50 ml of chloroform at room temperature. To this suspension was added Et_3N (0.54 g, 5.3 mmol), followed by Z-L-Asp(OBzl)-ONSu[2] (1.10 g, 2.4 mmol) with stirring. The mixture was stirred for 3 h and then kept standing overnight. A geratinous material appeared was dissolved by

adding 100 ml of chloroform with warming. The reaction mixture was washed successively with water, 1M HCl (1 M=1 mol dm^{-3}), a 5% sodium hydrogencarbonate solution, and water, and then concentrated under reduced pressure to leave a solid residue. The residue was triturated with ethyl acetate and collected by filtration. The residue was recrystallized from ethyl acetate. The data are given in Table 2.

Pentapeptide Esters (15—21). A typical run (**17** in Table 3) was as follows: Z-L-Asp(OBzl)-D-Ala-L-Val-L-Ala-L-Ala-OMe (**10**, 1.20 g) was dissolved in a mixture of acetic acid (45 ml) and water (10 ml), and hydrogenated in the presence of 5% Pd/C (0.50 g) with stirring at atmospheric pressure and 35°C for 5 h. The reaction mixture was filtered and the filtrate was concentrated to dryness under reduced pressure. The residue was dissolved in water and the solvent was evaporated under reduced pressure to remove the trace amount of remaining acetic acid. The procedure was repeated three times. The residue was recrystallized from water–acetone. The data are given in Table 3.

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