Structure-Taste Relationships of Aspartyl Tetrapeptide Esters

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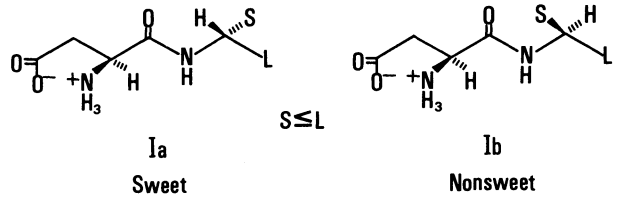
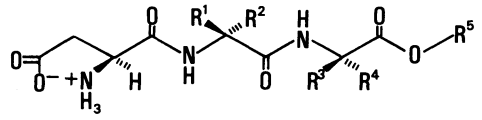
A series of fourteen analogues of L-α-Asp-Gly-Gly-Gly-OMe has been synthesized in relation to structural features of sweet peptides. The rule in the structure-taste relationships of tripeptides barely applies to the aspartyl tetrapeptide esters. In order for the aspartyl tetrapeptide esters to be sweet, the second amino acid must have a D-configuration and a small, compact alkyl side chain. However, the sweetness was accompanied by a bitter or astringent taste. Moreover, some of the tetrapeptides were not sweet but bitter, though they satisfied the requirements for sweet peptides. With increasing length of a peptide, it becomes difficult to fit the deep receptor pocket.

It has been suggested that sweet compounds have the AH-B-X[1](γ)[2] system in a molecule as a glucophore. In an attempt to elucidate structure-taste relationships, a large number of analogues of L-α-Asp-L-Phe-OMe[3] have been synthesized by several groups.[4-15] In previous papers,[16-18] the tastes of aspartyl dipeptides have been rationalized through projection formulas. In the structures (Ia and II), a small alkyl group (S or R^2) has been considered to participate in binding with the receptor through a hydrophobic interaction. The carboxyl and amino groups serve as a proton acceptor and a proton donor, respectively, in the hydrogen bonding with the receptor. The hydrophilic-hydrophobic balance in a molecule is another important factor. Space-filling properties are also important, since the receptor site seems to be in the shape of a deep pocket.[17] As described above, peptides with limited structures have shown sweetness, whereas various hydrophobic peptides are bitter. It has been known that peptides can be classified as bitter or nonbitter depending upon the magnitude of the hydrophobicity of their side chains.[19]



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).[16,18]



II

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

In an attempt to extend the general structure for sweet tripeptides to tetrapeptides, various aspartyl tetrapeptides were synthesized in the present study.

TABLE 1. PROTECTED TRIPEPTIDES

No.	Compound[a]	Yield %	TLC R_f[b]	Mp (θ_m/°C)	Recrys. solvent[c]	Appearance[d]	$[\alpha]_D^{25}$ Degree[e]	Formula[f]
1	Boc-Gly-Gly-Gly-OMe[g]	74.4	0.37	136—137	E	P		$C_{12}H_{21}O_6N_3$
2	Boc-D-Ala-L-Ala-L-Ala-OMe	76.1	0.67	108—110 (95)[h]	E-H	A	−37.4	$C_{15}H_{27}O_6N_3$
3	Boc-D-Ala-L-Ala-L-Val-OMe	83.7	0.76	152—153	E-H	N	−25.0	$C_{17}H_{31}O_6N_3$
4	Boc-D-Ala-L-Ala-L-Leu-OMe	84.6	0.70	117—118	E	N	−38.0	$C_{18}H_{33}O_6N_3$
5	Boc-D-Ala-L-Val-L-Ala-OMe	84.6	0.76	130—131	E-H	N	−27.3	$C_{17}H_{31}O_6N_3$
6	Boc-D-Ala-L-Val-L-Val-OMe	79.5	0.83	156—157	E-H	N	−16.2	$C_{19}H_{35}O_6N_3$
7	Boc-D-Val-L-Ala-L-Ala-OMe	54.6	0.80	163—164	E	A	−40.1	$C_{17}H_{31}O_6N_3$
8	Boc-D-Val-L-Ala-L-Val-OMe	79.6	0.82	155—156	E-H	N	−30.0	$C_{19}H_{35}O_6N_3$
9	Boc-D-Val-L-Val-L-Ala-OMe	79.0	0.83	146—148	E	A	−27.7	$C_{19}H_{35}O_6N_3$
10	Boc-D-Val-L-Val-L-Val-OMe	71.8	0.88	185—186	E	N	−18.9	$C_{21}H_{39}O_6N_3$
11	Boc-L-Ala-L-Ala-L-Ala-OMe[i]	74.0	0.68	186—188	E	N	−79.4	$C_{15}H_{27}O_6N_3$
12	Boc-L-Ala-L-Val-L-Ala-OMe[j]	84.5	0.71	177—178	E-H	N	−82.1	$C_{17}H_{31}O_6N_3$
13	Boc-L-Val-L-Ala-L-Ala-OMe	78.2	0.73	146—147	E-H	N	−75.4	$C_{17}H_{31}O_6N_3$
14	Boc-L-Val-L-Val-L-Ala-OMe	80.0	0.78	176.5—177.5	E-H	N	−79.8	$C_{19}H_{35}O_6N_3$

a) Abbreviations follow the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature in *J. Biol. Chem.*, **247**, 977(1972). b) TLC: $CHCl_3$:MeOH:AcOH=45:4:1 (v/v), detected with I_2 vapor. c) Recrystallization solvent: E, Ethyl Acetate; H, Hexane. d) Appearance: P, Prisms; A, Amorphous powder; N, Needles. e) In methanol, c=1.0%. f) 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. 8524). g) G.M. Bonora, C. Toniolo, V.N.R. Pillai, and M.Mutter, *Gazz. Chim. Ital.*, **110**, 503 (1980); mp 130—131°C. h) Softened. i) D. Le Nguyen, J.-R. Dormoy, B. Castro, and D. Prevot, *Tetrahedron*, **37**, 4229 (1981); mp 190°C, $[\alpha]_D^{20}$−69.3°(c 1, EtOH). j) See reference i); mp 173°C; $[\alpha]_D^{20}$−56.8°(c 1, EtOH).

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

Compd No.	X	Yield %	TLC R_f[a]	Mp (θ_m/°C)	Recrys. solvent[b]	Appearance[c]	$[\alpha]_D^{25}$ Degree[d]	Formula[e]
15	Gly-Gly-Gly-OMe	80.0	0.52	133—135	E-H	A	−4.6	$C_{26}H_{30}O_9N_4$
16	D-Ala-L-Ala-L-Ala-OMe	76.7	0.71	174—176	E	A	−21.4	$C_{29}H_{36}O_9N_4$
17	D-Ala-L-Ala-L-Val-OMe	72.2	0.78	156—158	E	N	−15.2	$C_{31}H_{40}O_9N_4$
18	D-Ala-L-Ala-L-Leu-OMe	88.6	0.73	124—126	E-H	A	−24.2	$C_{32}H_{42}O_9N_4$
19	D-Ala-L-Val-L-Ala-OMe	91.4	0.76	200—201 (180)[f]	D-E	N	−25.0	$C_{31}H_{40}O_9N_4$
20	D-Ala-L-Val-L-Val-OMe	80.9	0.80	186—187	E	N	−19.8	$C_{33}H_{44}O_9N_4$
21	D-Val-L-Ala-L-Ala-OMe	90.2	0.77	198.5—199.5	E	A	−17.9	$C_{31}H_{40}O_9N_4$
22	D-Val-L-Ala-L-Val-OMe	74.5	0.85	194.5—195.5	E	N	−9.9	$C_{33}H_{44}O_9N_4$
23	D-Val-L-Val-L-Ala-OMe	77.0	0.83	213.5—214.5	D-E	A	−22.4[g]	$C_{33}H_{44}O_9N_4$
24	D-Val-L-Val-L-Val-OMe	81.7	0.85	207—208	E	N	−13.4	$C_{35}H_{48}O_9N_4$
25	L-Ala-L-Ala-L-Ala-OMe	80.7	0.80	201—203.5	D-E-H	A	−38.5	$C_{29}H_{36}O_9N_4$
26	L-Ala-L-Val-L-Ala-OMe	73.4	0.88	227—228	D-E-H	A	−39.4	$C_{31}H_{40}O_9N_4$
27	L-Val-L-Ala-L-Ala-OMe	74.9	0.87	182—184	D-E-H	A	−37.1	$C_{31}H_{40}O_9N_4$
28	L-Val-L-Val-L-Ala-OMe	89.2	0.91	224—226	E-A	A	−44.1	$C_{33}H_{44}O_9N_4$

a) TLC: $CHCl_3$:MeOH:AcOH=45:4:1 (v/v), detected with I_2 vapor. b) Recrystallization solvent: E, Ethyl Acetate; H, Hexane; D, *N,N*-Dimethylformamide. c) Appearance: A, Amorphous powder; N, Needles. d) In acetic acid, 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. 8524). f) Softened. g) c=0.5%.

Table 3. Tetrapeptide esters, L-α-Asp-X

Compd No.	X	Yield %	TLC R_f[a]	Mp (θ_m/°C(dec))	Recrys. solvent[b]	Appearance[c]	$[\alpha]_D^{25}$ Degree[e]	Formula[e]
29	Gly-Gly-Gly-OMe	72.4	0.06	156.5—157.5	W-A	N	+28.9	$C_{11}H_{18}O_7N_4\cdot 2H_2O$
30	D-Ala-L-Ala-L-Ala-OMe	62.5	0.23	200—201	W	N	−25.6	$C_{14}H_{24}O_7N_4\cdot 1.5H_2O$
31	D-Ala-L-Ala-L-Val-OMe	70.5	0.32	201.5—202.5	W-A	A	−14.5	$C_{16}H_{28}O_7N_4\cdot 0.5H_2O$
32	D-Ala-L-Ala-L-Leu-OMe	57.8	0.31	187—188	W-A	N	−22.5	$C_{17}H_{30}O_7N_4\cdot 0.75H_2O$
33	D-Ala-L-Val-L-Ala-OMe	65.1	0.36	213—214	W	N	−22.3	$C_{16}H_{28}O_7N_4\cdot 2H_2O$
34	D-Ala-L-Val-L-Val-OMe	67.8	0.43	205—206	W	N	−11.8	$C_{18}H_{32}O_7N_4\cdot 3H_2O$
35	D-Val-L-Ala-L-Ala-OMe	80.2	0.34	210—211	W	N	−22.3	$C_{16}H_{28}O_7N_4\cdot 3.5H_2O$
36	D-Val-L-Ala-L-Val-OMe	76.0	0.39	227—228	W	N	−13.2	$C_{18}H_{32}O_7N_4\cdot 0.5H_2O$
37	D-Val-L-Val-L-Ala-OMe	69.9	0.45	>240	W	N	−21.8[f]	$C_{18}H_{32}O_7N_4\cdot H_2O$
38	D-Val-L-Val-L-Val-OMe	71.4	0.50	>240	W	N	−1.6[g]	$C_{20}H_{36}O_7N_4\cdot H_2O$
39	L-Ala-L-Ala-L-Ala-OMe[h]		0.16	paste				
40	L-Ala-L-Val-L-Ala-OMe	70.2	0.38[i]	227—228	W	A	−57.6[g]	$C_{16}H_{28}O_7N_4\cdot H_2O$
41	L-Val-L-Ala-L-Ala-OMe	69.5	0.34	212—214	W	N	−94.6	$C_{16}H_{28}O_7N_4\cdot 1.5H_2O$
42	L-Val-L-Val-L-Ala-OMe	61.3	0.49[f]	>240	W	A	−50.0[g]	$C_{18}H_{32}O_7N_4\cdot H_2O$

a) TLC: $CHCl_3$:MeOH:AcOH:H_2O=32:15:1:3 (v/v), detected with ninhydrin. b) Recrystallization solvent: W, Water; A, Acetone. c) Appearance: A, Amorphous powder; N, Needles. d) In water, 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. 8524). f) c=0.5. g) In acetic acid, c=0.5%. h) Not analyzed. i) *n*-BuOH:AcOH:H_2O=4:1:1 (V/V).

Another interesting problem is to examine whether the above rule for bitter peptides can be applied to tetrapeptides with a possible sweet structure in their molecules.

Synthesis

Synthesis of Boc-dipeptide esters and the removal of the Boc group were carried out according to the method described in a previous paper.[18] The protected tripeptides in Table 1 were prepared by condensation of the appropriate dipeptide ester with a Boc-amino acid *N*-hydroxysuccinimide ester. The Boc group of the tripeptide esters was removed with *p*-toluenesulfonic acid (TosOH) in methanol.[20] The protected tetrapeptides in Table 2 were prepared by condensation of *N*-benzyloxycarbonyl-L-aspartic acid β-benzyl α-succinimido ester (Z-L-Asp(OBzl)-ONSu[18]) with the appropriate tetrapeptide ester. The desired tetrapeptide esters in Table 3 were obtained by deprotection of the benzyloxycarbonyl and benzyl groups from the protected tetrapeptides in Table 2 by hydrogenation over Pd/C.

Results and Discussion

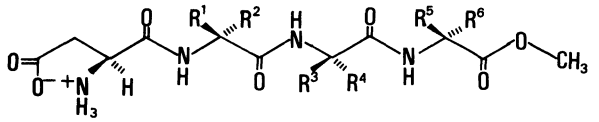
In order to evaluate the contributions of the side chains of each amino acid, the substituents R^1—R^6 of III (Fig. 3) were systematically modified. The results are summarized in Table 4. In order for aspartyl tripeptides to be sweet, the second amino acid must

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

Compd No.	X	R^1	R^2	R^3	R^4	R^5	R^6	Taste[b]
29	Gly-Gly-Gly-OMe	H	H	H	H	H	H	0
30	D-Ala-L-Ala-L-Ala-OMe	H	Me	H	Me	Me	H	0.5
31	D-Ala-L-Ala-L-Val-OMe	H	Me	H	Me	*i*-Pr	H	−
32	D-Ala-L-Ala-L-Leu-OMe	H	Me	H	Me	*i*-Bu	H	−
33	D-Ala-L-Val-L-Ala-OMe	H	Me	H	*i*-Pr	Me	H	2
34	D-Ala-L-Val-L-Val-OMe	H	Me	H	*i*-Pr	*i*-Pr	H	−
35	D-Val-L-Ala-L-Ala-OMe	H	*i*-Pr	H	Me	Me	H	+
36	D-Val-L-Ala-L-Val-OMe	H	*i*-Pr	H	Me	*i*-Pr	H	−
37	D-Val-L-Val-L-Ala-OMe	H	*i*-Pr	H	*i*-Pr	Me	H	5(−)
38	D-Val-L-Val-L-Val-OMe	H	*i*-Pr	H	*i*-Pr	*i*-Pr	H	−
39	L-Ala-L-Ala-L-Ala-OMe	Me	H	H	Me	Me	H	−
40	L-Ala-L-Val-L-Ala-OMe	Me	H	H	*i*-Pr	Me	H	0
41	L-Val-L-Ala-L-Ala-OMe	*i*-Pr	H	H	Me	Me	H	0
42	L-Val-L-Val-L-Ala-OMe	*i*-Pr	H	H	*i*-Pr	Me	H	0

a) For structures, see Fig. 3. b) Times sucrose (weight basis, sucrose=1). 0=tasteless: −=bitter; +=faintly sweet; (−)=sweet-bitter.

have a D-configuration and a small alkyl side chain.[18] Therefore, in systematic structural variation of the tetrapeptides (III), a small alkyl group was introduced at R^2 so as to meet the sweet structure (II). An L-antipode was introduced for the third amino acid, because the modification of tripeptides showed that an L-configuration of the third amino acid was required for a potent sweet taste.



III

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

As the first step, L-α-Asp-Gly-Gly-Gly-OMe (**29**) was synthesized. The peptide (**29**) was tasteless. The first candidate for sweet tetrapeptides was L-α-Asp-D-Ala-L-Ala-L-Ala-OMe (**30**). As expected, it was slightly sweet, 0.5 times as sweet as sucrose (Table 4). Replacement of a methyl group at R^5 of **30** by an isopropyl or isobutyl group gave L-α-Asp-D-Ala-L-Ala-L-Val-OMe (**31**) and L-α-Asp-D-Ala-L-Ala-L-Leu-OMe (**32**), respectively, which were weakly bitter. Replacement of a methyl group at R^4 of **30** by an isopropyl group gave a sweet peptide, L-α-Asp-D-Ala-L-Val-L-Ala-OMe (**33**). When a methyl group at R^5 of **33** was replaced by an isopropyl group, the resulting peptide, L-α-Asp-D-Ala-L-Val-L-Val-OMe (**34**), was bitter

Next, the second D-Ala was replaced by D-Val. The resulting peptides were faintly sweet (**35**), weakly bitter (**36** and **38**), and sweet-bitter (**37**).

Finally, the required configuration (R^1=H; R^2= small alkyl group) at the second amino acid was confirmed by synthesizing several tetrapeptides having an opposite configuration at this chiral center (**39**—**42**). All of them were devoid of sweetness. Thus, the requirement was confirmed.

In conclusion, the rule in the structure-taste relationships of tripeptides barely applies to the sweet tetrapeptides. However, the sweetness was accompanied by a bitter or astringent taste. Moreover, some of the tetrapeptides were not sweet but bitter, though they satisfied the requirements for the sweet peptides. The taste of aspartyl tetrapeptide esters tends to follow Ney's rule for bitter peptides. With increasing length of a peptide, it becomes difficult to fit the narrow receptor pocket. This may be a major reason that some of the tetrapeptides were not sweet but bitter, though they satisfied the requirements for sweet peptides. A similar result has been obtained in the modification of tripeptides,[18] in which the sweetness potencies significantly fell off when sweet dipeptides have been lengthen to tripeptides. Accordingly, the results obtained for the tetrapeptides, in conjunction with the results of di-[16] and tripeptides,[18] support that the receptor site is in the shape of a deep pocket with the binding site inside it.[17]

Experimental

All 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. TLC was performed on precoated silica gel $60F_{254}$ plates (E. Merck) and spots were detected with ninhydrin or I_2 vapor. All compounds were essentially homogeneous on TLC. Sweetness potency was not evaluated by the panel method due to the small quantities available. In general, a panel evaluation needs a large quantity of sample. The yield, after purification, of each peptide (Table 3) was less than 0.8 g. The quantity of tetrapeptides obtained here was insufficient for the panel evaluation. Therefore, sweetness evaluation was carried out by the author by matching a threshold concentration of each compound with that of sucrose. Test solutions of the tetrapeptides were prepared at several concentrations. A series of the solutions was tasted up and down. Thus, it was possible to reproducibly determine the concentration which matched a 0.6% aqueous solution of sucrose. Therefore, it has been considered that the sweetness potencies in Table 4 are reproducible and reliable

enough to discuss the functions of a small hydrophobic group.

Materials. Boc-amino acids were purchased from Peptide Institute Inc. Esters of Gly and L-Val were synthesized in our laboratory. Z-L-Asp(OBzl)-OH and esters of L-Ala and L-Leu were purchased from Kokusan Chemical Works Ltd.

Protected Tripeptide Esters (1—14). A typical run (**6** in Table 1) was as follows: To a solution of Boc-L-Val-L-Val-OMe[21] (2.31 g, 7 mmol) in 20 ml of methanol was added TosOH·H_2O (1.60 g, 8.4 mmol). The mixture was stirred at 30°C for 5 h. The solvent was evaporated under reduced pressure at a bath temperature of 50°C to give H-L-Val-L-Val-OMe·TosOH as an oily residue. The oil was dissolved in 40 ml of chloroform and cooled in an ice-bath. To this solution was added triethylamine (Et_3N, 0.85 g, 8.4 mmol), followed by Boc-D-Ala-ONSu (1.72 g, 7 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 an oily product. The oil was crystallized from ethyl acetate–hexane to give Boc-D-Ala-L-Val-L-Val-OMe (**6**) as needles. Recrystallization was carried out from the same solvent. The data are given in Table 1.

Protected Tetrapeptide Esters (15—28). A typical run (**20** in Table 2) was as follows: To a solution of Boc-D-Ala-L-Val-L-Val-OMe (**6**, 1.73 g, 4.3 mmol) in 20 ml of methanol was added TosOH·H_2O (1.00 g, 5.3 mmol). The mixture was stirred at 30°C for 5 h. The solvent was evaporated under reduced pressure at a bath temperature of 50°C to give H-D-Ala-L-Val-L-Val-OMe·TosOH as a solid residue. The residue was dissolved in 30 ml of chloroform and cooled in an ice-bath. To this solution was added Et_3N (0.53 g, 5.3 mmol), followed by Z-L-Asp(OBzl)-ONSu[18] (1.82 g, 4 mmol) with stirring. The mxiture was stirred for 3 h and then kept standing overnight. The reaction mixture was washed successively with water, 1 M HCl (1 M=1 mol dm^{-3}), a 5% sodium hydrogencarbonate solution and water, and then concentrated under reduced pressure to leave an oily product. The oil was crystallized from ethyl acetate–hexane to give Z-L-Asp(OBzl)-D-Ala-L-Val-L-Val-OMe (**20**) as needles. Recrystallization was carried out from the same solvent. The data are given in Table 2.

Tetrapeptide Esters (29—42). A typical run (**34** in Table 3) was as follows: Z-L-Asp(OBzl)-D-Ala-L-Val-L-Val-OMe (**20**, 1.60 g) was dissolved in a mixture of acetic acid (40 ml) and water (10 ml), and hydrogenated in the presence of 5% Pd/C (0.45 g) with stirring at atmospheric pressure and room temperature 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 four times. The crystalline residue thus obtained was recrystallized from water to give 0.80 g (67.8%) of L-α-Asp-D-Ala-L-Val-L-Val-OMe·3H_2O (**34**) as needles. The data are given in Table 3.

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