Journal of Peptide Science, Vol. 3, 231–241 (1997)

Conformational Analysis of the Dipeptide Taste Ligand L-Aspartyl-D-2-aminobutyric acid-(*S*)-α-ethylbenzylamide and its Analogues by NMR Spectroscopy, Computer Simulations and X-ray Diffraction Studies

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Received 31 August 1996
Accepted 11 October 1996

Abstract: A dipeptide taste ligand L-aspartyl-D-2-aminobutyric acid-(*S*)-α-ethylbenzylamide was found to be about 2000 times more potent than sucrose. To investigate the molecular basis of its potent sweet taste, we carried out conformational analysis of this molecule and several related analogues by NMR spectroscopy, computer simulations and X-ray crystallographic studies. The results of the studies support our earlier model that an L-shape molecular array is essential for eliciting sweet taste. In addition, we have identified an aromatic group located between the stem and the base of the L-shape, which is responsible for enhancement of sweetness potency. In this study, we also assessed the optimal size of the essential hydrophobic group (X) and the effects of the chirality of the second residue toward taste. ©1997 European Peptide Society and John Wiley & Sons, Ltd.

J. Pep Sci. 3: 231–241, 1997

(No. of Figures: 11. No. of Tables: 7. No. of Refs: 14)

Keywords: conformational analysis; NMR spectroscopy; X-ray diffraction; peptide-based taste ligands; artificial sweeteners; computer simulations

Introduction

The transduction of taste is believed to be initiated by formation of a host–guest complex between taste ligands and receptor proteins located on the surface of the taste cell. Since no taste receptors have been isolated to date, extensive studies have been focused on the various ligands in order to probe the molecular basis of taste. Comparisons among a wide variety of sweet compounds reveal common structural features. Shallenberger and Acree proposed the existence of a hydrogen bond donor (AH) and a hydrogen bond acceptor (B) in sweet molecules [1]. It was postulated that the AH and B groups form complementary intermolecular hydrogen bonds with equivalent H-bond acceptor functionalities on the receptor molecules. Further studies have suggested that in addition to the AH and B groups, a hydrophobic region (X) in a ligand is necessary to elicit sweet taste of high potency [2].

The discovery of aspartame [3], a dipeptide with potent sweet taste (approximately 200 times more

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CCC 1075-2617/97/030231-11

potent than sucrose), stimulated extensive structure–activity relationship studies [4, 5]. The structure of aspartame is Asp-Phe-OMe, where the protonated amino and carboxylate groups of the N-terminal aspartic acid residue have been proposed to be the AH and B groups of the Shallenberger and Acree model, respectively. The aromatic side chain of the phenylalanine residue has been suggested to be the hydrophobic moiety X. Structural modifications to aspartame suggest that in addition to having the necessary glucophores, it is also critical for a sweet ligand to adopt an appropriate three-dimensional structure. Through extensive conformational studies of aspartame and related analogues using X-ray crystallography, ^{1}H-NMR, and molecular mechanics calculations, we have developed models describing the molecular arrays responsible for sweet and bitter taste [6]. According to our model, the overall topology of a sweet tasting molecule can be described as an 'L'-shape structure with the aspartyl moiety forming the stem of the 'L' and the hydrophobic group X forming the base of the 'L.' The zwitterionic ring of the aspartyl residue is coplanar and essentially perpendicular to the X group. Figure 1 shows the superposition of the 'L'-shape model and an 'L'-shaped structure for aspartame.

It was recently reported that a peptide-based taste ligand Asp-D-2-amino butyric acid-(*S*)-α-ethylbenzylamide (**I**) is about 2000 times more potent than sucrose [7]. A similar analogue Asp-D-2-amino butyric acid-(*S*)-α-methylbenzylamide (**II**) is about 360 times more potent than sucrose [7]. However, another closely related analogue with stereochemical variation at the C-terminus, Asp-D-Ala-(*R*)-α-methylbenzylamide (**III**), is slightly bitter. Since the C-terminal moiety of the aforementioned molecules functions as the hydrophobic group (X), conformational analyses of these molecules should provide information about the effects of the three-dimensional structures of the C-terminal residue on taste properties. In addition to the molecules mentioned above, our studies also include three additional analogues. Among these analogues, aspartyl-(*R*)-1,1-diaminopropyl-(*S*)-α-phenylpropionyl (**IV**) is an analogue of compound **II** with a retro-inverso modification of the C-terminal amide bond. This compound was found to be faintly sweet with a slight bitter aftertaste. Aspartyl-α-aminocyclopentane carboxylic acid-(*S*)-α-ethylbenzylamide (**V**) is an analogue of compound **I** with the second residue being an achiral aminocyclopentyl acid (Ac^{5}c) in place of the D-Abu residue. This compound was found to be about 1000 times sweeter than sucrose. Aspartyl-diethylglycine-(*S*)-α-ethylbenzylamide (**VI**) is also an analogue of compound **I** with the C_α proton of the second residue substituted by an ethyl group, resulting in an achiral residue at the second position. This compound was found to be about 500 times more potent than sucrose. The structures of all six molecules are shown in Figure 2.

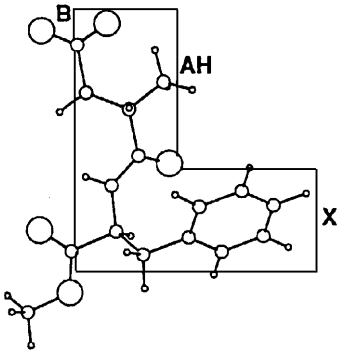

Figure 1 The topological array of aspartame superimposed with the 'L'-shape model.

In the current study, a combination of ^{1}H-NMR, computer simulations as well as X-ray crystallography was carried out to derive the minimum energy three-dimensional conformations of the molecules. The conformational features of these molecules were then correlated to their taste properties in an effort to probe the molecular basis of taste of dipeptide-like sweeteners.

Materials and Methods

Materials

Compounds **I**, **II** and **III** were synthesized by L. L. D'Angelo, G. A. King III and J. G. Sweeny of the Coca-Cola Company. Compounds **V** and **VI** were synthesized at the Ajinomoto Company in Japan. Compound **IV** was synthesized in our laboratories.

NMR Spectroscopy

A series of one-dimensional and two-dimensional ^{1}H-NMR experiments including double-quantum-filtered COSY (DQF-COSY) [8] and rotating frame nuclear Overhauser experiments (ROESY) [9, 10] were carried out to derive structural information about the molecules in solution. All spectra were recorded on a Bruker AMX 500 spectrometer at 300 K. Samples were prepared in DMSO-d_6 at

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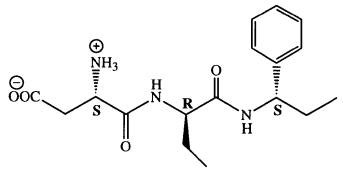
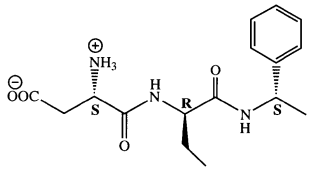


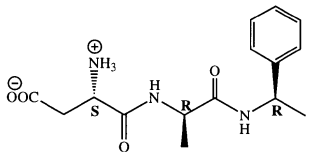
Compound I

L-Aspartyl-(*R*)-α-aminoisobutyric acid-(*S*)-α-ethylbenzyl amide [Asp-D-Abu-(*S*)-α-EBA]



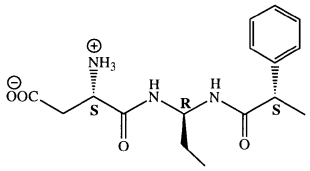
Compound II

L-Aspartyl-(*R*)-α-aminoisobutyric acid-(*S*)-α-methylbenzyl amide [Asp-D-Abu-(*S*)-α-MBA]



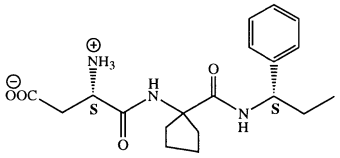
Compound III

L-Aspartyl-D-alanine-(*R*)-α-methylbenzyl amide [Asp-D-Ala-(*R*)-α-MBA]



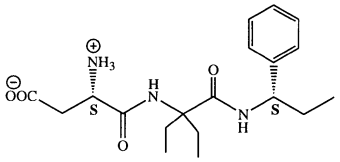
Compound IV

L-Aspartyl-(*R*)-1,1-diaminopropyl-(*S*)-α-phenylpropionyl [Asp-(R)-*g*Abu-(*S*)-α–PhProp]



Compound V

L-Aspartyl-α-aminocyclopentane carboxylic acid-(*S*)-α-ethylbenzyl amide [Asp-Ac^5c-(*S*)-α-EBA]



Compound VI

L-Aspartyl-diethylglycine-(*S*)-α-ethylbenzyl amide [Asp-Deg-(*S*)-α-EBA]

Figure 2 The structures of compounds involved in our studies.

concentrations between 30 and 40 mM. The resonance of DMSO-d_6 ($\delta = 2.49$ p.p.m.) was used as an internal standard. The one-dimensional spectra contain 16 K data points with spectral widths of ± 2500 Hz. The two-dimensional double-quantum-filtered COSY (DQF-COSY) experiments were acquired with 2 K data points in the t2 domain and 256 points in the t1 domain. The rotating frame nuclear Overhauser experiments (ROESY) were acquired with mixing time of 150 ms and with a spin locking field of 2.5 kHz. The spectra were obtained using 2K data points in the t2 domain and 512 points in the t1 domain. Applying the zero filing procedure to the f1 domain resulted in a final matrix of 2 K × 2 K data points. Multiplication with a phase-shifted sine function was used to enhance the spectra.

X-ray Diffraction Analysis

Colourless single crystals of compound **I** [Asp-D-Abu-(*S*)-α-EBA] were obtained by slow evaporation of methanol/H_2O. Unit cell parameters were determined by least-squares refinement of the setting angles of 25 high angle reflections ($16 < \theta < 30°$). Compound **I** crystallizes in the monoclinic system, space group $P2_1$ with $a = 10.585(9)$, $b = 4.820(3)$, $c = 21.265(2)$ Å, $\beta = 95.14(4)°$ and $Z = 2$. An Enraf–Nonius automated diffractometer at the Biocrystallography Research Center of the CNR at the Chemistry Department of the University of Napoli 'Federico II' was employed for data collection, with graphite-monochromated CuKα radiation ($\lambda = 1.54178$ Å). The structure was solved by direct methods using the SIR 92 program [11]. The best E maps revealed most of the non-H atoms. The remaining atoms and the O atoms of the co-crystallized water molecules were found from subsequent Fourier syntheses. At the end of the isotropic refinement, hydrogen atoms were in part located in successive Fourier maps, and in part calculated in their stereochemically expected positions. Refinement of the structure was performed by a full matrix least-squares procedure minimizing the quantity $\Sigma w(F_o - F_c)^2$, with $w = 1/\sigma(F_o)^2$. All non-H atoms were refined anisotropically. H atoms were introduced in the calculations with isotropic thermal factors equal to the B_{eq} of the carrier atom and their parameters were not refined. Final R and R_w values were 0.065 and 0.067 for the 1788 observed reflections [$I > 3\sigma(I)$]. In the final difference Fourier synthesis the maximum and minimum electron densities were 0.257 and −0.283 eÅ^3. The scattering factors for all atomic species were calculated according to the method of Cromer and Waber [12]. Tables of atomic coordinates and anisotropic thermal factors for non-hydrogen atoms and isotropic thermal parameters for hydrogen atoms, bond lengths, bond angles and torsion angles have been deposited

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as Supplementary Material with the Cambridge Crystallographic Data Bank.

Computer Simulations

All computer simulations were performed on Silicon Graphics personal Iris 4D-25 workstations and Challenge L computers. Conformational search and molecular mechanics calculations were carried out using the InsightII molecular modelling package and the Discover force field program [13]. A distance-dependent dielectric constant was used for all calculations. Energy minimizations were achieved by using the steepest descent algorithm for 200 steps followed by the VA09A algorithm until all the derivatives were less than 0.001 kcal/molÅ.

Conformational searches were carried out by applying the grid search method. Previous studies have shown that in all of the L-aspartyl-based analogues, the aspartic acid residue shows similar conformational preferences independent of the structure and configuration of the second residue [6]. The side chain conformation of the aspartic acid between the C_α and C_β bond ($\chi_1{}^1$) is always g^- and the torsional angle between the C_α and CO bond of the residue aspartic acid (ψ^1) is between 100° and 160°. Therefore, in our conformational search process, the values of $\chi_1{}^1$ and ψ^1 were assigned to −60° and 140°, respectively. Only three of the backbone torsional angles were involved in the grid search, including the ϕ^2, ψ^2 torsions of the second residue and a ϕ^3 torsion. Figure 3 shows the definition of these torsion angles. Each one of the three torsions was varied at 30° increments, resulting in 1728 structures for each molecule studied. These structures were then minimized and clustered into families according to their structural similarities. Finally, the structures not consistent with the data derived from NMR experiments were discarded. The remaining structures were then considered in our conformational studies to explore the molecular basis of taste for dipeptide-like sweeteners.

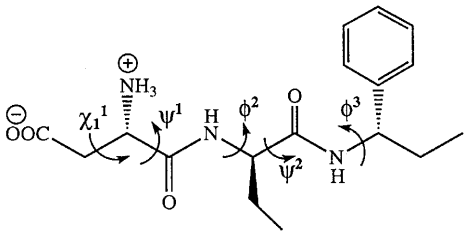

Figure 3 Definition of the torsion angles which are used to describe the conformations of the molecules in our studies.

Results and Discussion

Figure 4 shows the stereo drawing of the molecular model of **I** [Asp-D-Abu-(*S*)-α-EBA] as determined by X-ray diffraction. The molecule crystallizes as trihydrate and, as usually observed in dipeptides of the aspartame family, the N-terminal aspartyl moiety exists as a zwitterion, with the N-terminal amino group positively charged and the Asp side-chain carboxylate group negatively charged. The L-aspartyl residue assumes a conformation very similar to that observed in other aspartame dipeptide analogues [6] , where on the average the dihedral angles ψ^1, ω^1, $\chi_1{}^1$ and $\chi_{2,1}$ show values of +156°, +175°, −69° and −173° (or +7° for $\chi_{2,2}$), respectively. These conformational angles in compound **I** have values of +145.8°, +175.1°, −71.2°, and 165.9°. The conformation about the Asp C^α–C^β bond ($\chi_1{}^1$) is gauche$^-$ (g^-). The carboxylate of the aspartyl side chain is nearly coplanar with the C^α–C^β bond: the $\chi_{2,1}$ and $\chi_{2,2}$ dihedral angles are close to 180° and 0°, respectively.

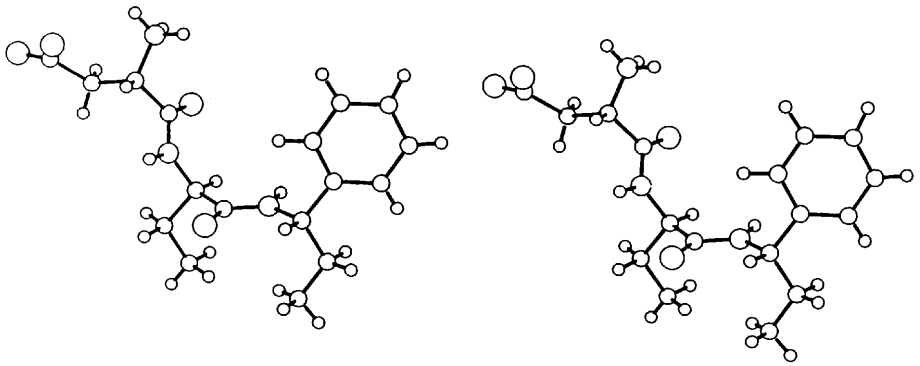

Figure 4 Stereo view of the molecular model of **I** [Asp-D-Abu-(*S*)-α-EBA] as derived from X-ray diffraction.

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In dipeptides of the aspartame family the conformation of the second residue varies greatly, depending upon the configuration and the conformational constraints of the molecules. In the present case the molecule has a second residue with the D-configuration. The D-Abu residue adopts a semi-extended conformation characterized by dihedral angles $\varphi^2 = 125.3^\circ$ and $\psi^2 = -115.4^\circ$. As shown in Figure 4 the bulkier hydrophobic group represented by the C-terminal amide group adopts a conformation in which it constitutes the base of an 'L'-shape extending along the $+x$ direction, whereas the stem of the L is roughly represented by the Asp-Abu moiety, which extends along the $+y$ direction.

For aspartame analogues the conformation observed in the crystalline state is among the minimum energy conformations allowed to the molecule and packing forces, such as electrostatic interactions between charged groups and hydrogen bonds, to a greater extent and van der Waals and hydrophobic interactions (stacking of aromatics or alkyl–phenyl and alkyl–alkyl interactions) to a lesser extent, greatly influence and determine the observed conformation. In the crystal the molecules of compound **I** pack in such a way that the hydrophilic zwitterions of the aspartyl moieties of symmetry-related molecules face each other in the unit cell interacting also with the co-crystallized water molecules though electrostatic forces and hydrogen bonds. In the crystal the water molecules occupy a channel along the *b* direction.

The coupling constants and NOE data of all molecules measured from the NMR spectra are listed in Table 1. The NOE's were obtained from the ROESY experiments and are qualitatively classified according to their relative intensities (i.e. strong (s); medium (m) and weak (w)). The coupling constant values were obtained from the 1D and 2D DQF-COSY spectra.

For compound **I** [Asp-D-Abu-(*S*)-α-EBA], five families of conformations were found in solution with energies not higher than 5 kcal/mol from the lowest energy conformer. Figure 5(A) and (B) shows these conformations from two different view angles. The axes of the coordinate system are marked accordingly in the figures. The torsion angles of these conformers are listed in Table 2, along with their relative energies. Among the preferred conformations of compound **I**, conformers 1, 2 and 3 are characterized by an 'L'-shape array where the C-terminal of the molecule forms the base of the 'L'. The conformation of compound **I** in the crystalline

Table 1 Coupling Constants and NOE Data Measured from NMR Experiments

Compound	I[a]	III	IV	V	VI
$J_{\alpha\beta l}$(Asp), Hz	4.49	3.82	3.06	5.20	5.18
$J_{\alpha\beta h}$ (Asp), Hz	9.32	9.01	8.54	7.50	7.84
$J_{\alpha\beta l}$ (D-Abu), Hz	4.89				
$J_{\alpha\beta h}$ (D-Abu), Hz	3.30				
$J_{NH\text{-}H\alpha}$ (D-Abu), Hz	8.56	7.94	7.34	8.25	8.26
NOE ($H_\alpha{}^1$–$H_{\beta l}{}^1$)	s	m	m	s	s
NOE ($H_\alpha{}^1$–$H_{\beta h}{}^1$)	m	w	w	s	m
NOE ($H_\alpha{}^2$–$H_\beta{}^2$)			s	s	
NOE ($H_\alpha{}^2$–$_{\beta l}{}^2$)	s				
NOE ($H_\alpha{}^2$–$H_{\beta h}{}^2$)	s				
NOE ($H_\alpha{}^2$–$H_\gamma{}^2$)			m		
NOE ($H_\alpha{}^2$–$H_{\beta l}{}^1$)		w			
NOE ($H_\alpha{}^3$–$H_\beta{}^3$)	s	s	m	s	s
NOE (HN^3–$H_\alpha{}^3$)	m		s	s	m
NOE (HN^3–$H_\alpha{}^2$)		s	m		
NOE (HN^3–$H_\beta{}^2$)		m			
NOE (HN^3–$H_\gamma{}^2$)		w			
NOE (HN^2–$H_\alpha{}^2$)		w			
NOE (HN^2–$H_\alpha{}^2$&HN^3–$H_\alpha{}^2$)	s				
NOE (HN^2–$H_\alpha{}^1$)	m		w	s	
NOE (HN^3–$H_\alpha{}^1$)				w	
NOE (HN^3–$H_\beta{}^3$)	m	m		s	
NOE ($H_{\phi l}{}^3$–$H_\alpha{}^3$)	s			s	s
NOE ($H_{\phi h}{}^3$–$H_\alpha{}^3$)	w				
NOE (HN^3–$H_\phi{}^3$)			m	m	m
NOE ($H_\phi{}^3$–$H_\alpha{}^3$)			m		
NOE ($H_{\phi h}{}^3$–$H_\beta{}^3$)	s				
NOE ($H_{\phi l}{}^3$–$H_\beta{}^3$)	w				

[a]The NMR spectrum for compound **II** is essentially identical to that of compound **I**.

Table 2 Preferred Conformation of Compound **I** in Solution

	$\chi_1{}^1$	ψ^1	ϕ^2	ψ^2	ϕ^3	Energy (kcal/mol)
Conformer 1	−59	133	77	−90	−78	0.00
Conformer 2	−59	145	75	47	−80	1.34
Conformer 3	−58	137	78	−143	70	3.74
Conformer 4	−59	144	−64	59	−81	4.20
Conformer 5	−59	143	70	50	70	4.38

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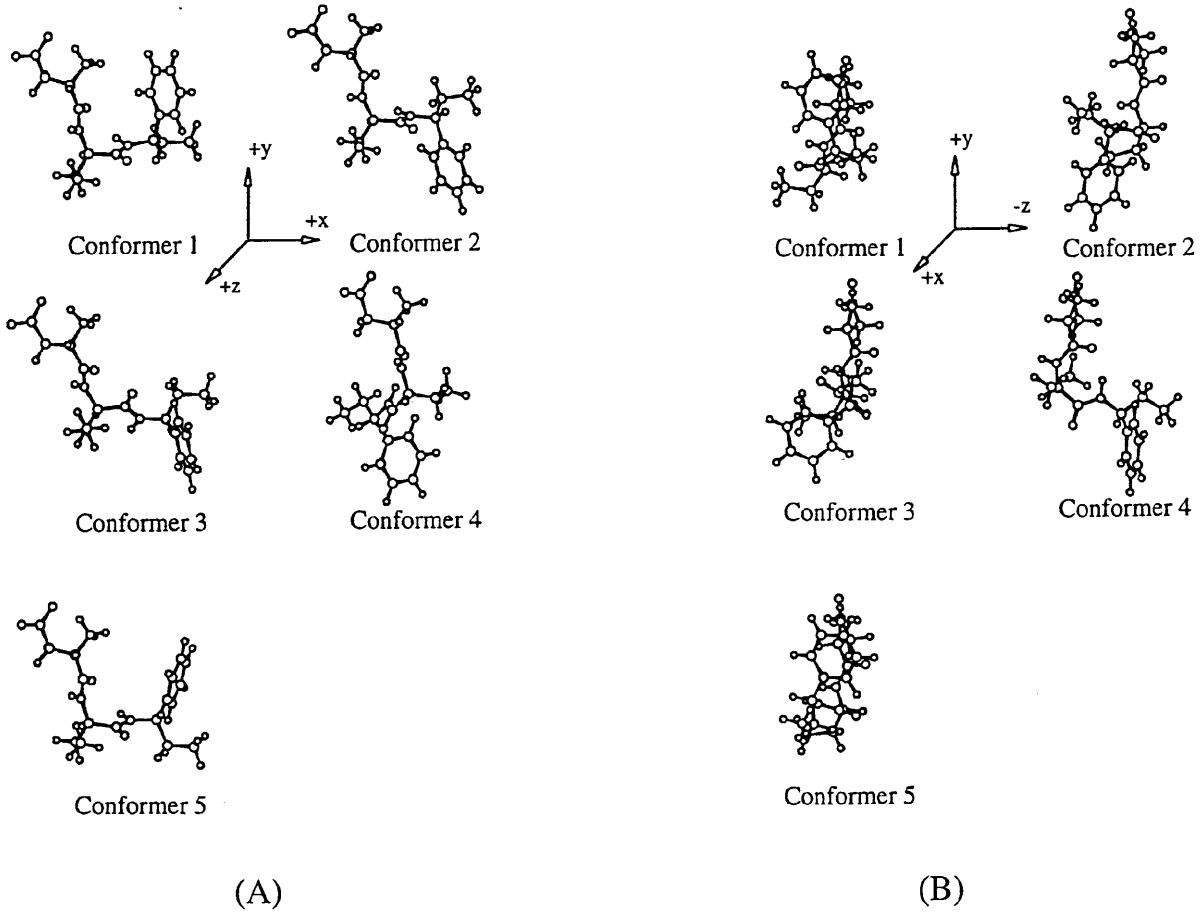

Figure 5 The preferred conformations of compound **I** [Asp-D-Abu-(*S*)-α-EBA] in solution: (A) view 1; (B) view 2.

state has been subsequently optimized using energy minimizations. The two conformations are compared in Figure 6(A) and (B).

The overall topology of the minimized X-ray conformation is very similar to the lowest energy conformation (conformer 1) found in solution. In this conformation, the phenyl ring is located above the base of the 'L' and is coplanar with the 'L'-shape array. Figure 7 shows a comparison of conformer 1 and the most preferred conformation of a sweet analogue Asp-Ac^{3}c-OnPr [14], which was studied earlier in our laboratories. The results suggest that the phenyl ring of the compound **I** is not required for sweet taste. However, the highly potent sweetness of the compound suggests that there might be an additional binding zone in the sweetener receptor which interacts with the phenyl ring positioned between the stem and the base of the 'L'. This interaction provides great enhancement to the sweetness potency of the compound.

Compound **II** [Asp-D-Abu-(*S*)-α-MBA] has one less C-terminal methylene group than compound **I** and the result of the computer simulations showed that the conformational preferences of both molecules are similar. Like compound **I** [Asp-D-Abu-(*S*)-α-EBA], compound **II** also has five families of low-energy conformations. These conformations can be found in the supplementary materials. The torsion angles and relative energies of the conformations are listed in Table 3. Previous studies have shown that the sweetness potency of L-aspartyl based dipeptide derivatives is dependent on the length and size of the hydrophobic group (X). The fact that compound **I** has higher sweetness potency than compound **II** is an indication that the X group of compound **I** is closer to the optimal size than that of compound **II**.

Five families of low-energy conformations were found for compound **III** [Asp-D-Ala-(*R*)-α-MBA]. These conformations are displayed in Figure 8(A) and (B). The torsion angles and the relative energies

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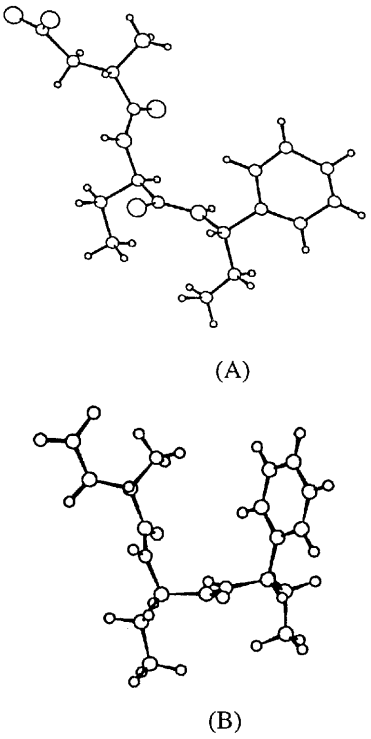

Figure 6 (A) Conformation of compound **I** [Asp-D-Abu-(*S*)-α-EBA] in crystalline state (A) determined by X-ray diffraction studies and (B) after energy minimization.

of the conformers are listed in Table 4. Similar to compound **I** [Asp-D-Abu-(*S*)-α-EBA], the three lowest energy minima of compound **III** are characterized by 'L'-shape conformations. However, in the lowest energy conformer 1, the phenyl ring points downward from the base of 'L'. In the second lowest energy conformer 2, the phenyl ring projects toward the $-z$ direction, which should lead to a bitter taste according to the models proposed earlier. Conformer 3 is similar to conformer 1 of compound **I**, which corresponds to a sweet taste-enhancing conformation. The fact that compound **III** is slightly bitter suggests that the topology of conformer 1 does not correspond to a sweet tasting conformation. This further suggests that the displacement of the phenyl ring beneath the base of 'L' interferes with the binding of the ligand with the taste receptor, resulting in a loss of sweetness.

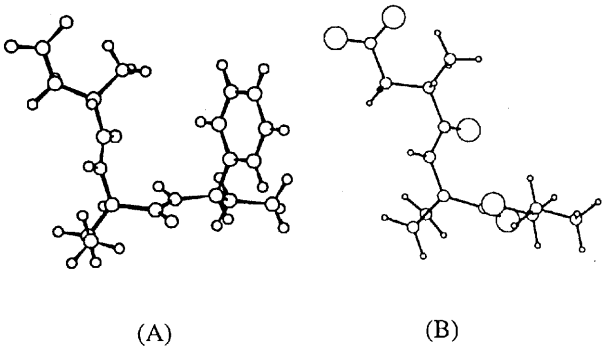

Figure 7 Comparison of the lowest energy conformations of compound **I** [Asp-D-Abu-(*S*)-α-EBA] and L-Asp-Ac^{3}c-OnPr (B).

Three families of low energy conformations were found for compound **IV** [Asp-(*R*)-gAbu-(*S*)-α-PhProp]. These conformations are shown in Figure 9(A) and (B). The torsion angles and relative energies of the conformations are shown in Table 5. Conformer 1 looks L-shaped from view 1. However, from view 2 it is clear that the hydrophobic group projects towards the $+z$ axis. In conformer 2, the hydrophobic group and the zwitterionic ring are extended from each other. Conformer 3 is reverse 'L'-shaped. All three conformations correspond to topochemical arrays incapable of ligand receptor binding, according to models proposed in our earlier studies [6]. Overall, the retro-inverso modification between the second and third residues changed the conformational preferences of the molecule and resulted in distorted 'L'-shaped conformations. This should account for the loss of sweet taste of the compound.

Nine low-energy conformational families were found for compound **V** [Asp-Ac^{5}c-(*S*)-α-EBA]. These conformations can be found in the supplementary materials. The torsion angles and the relative energies are listed in Table 6. Modification at the

Table 3 Preferred Conformation of Compound **II** in Solution

	χ_1^1	ψ^1	χ^2	ψ^2	ϕ^3	Energy (kcal/mol)
Conformer 1	−59	134	77	−91	−77	0.00
Conformer 2	−59	145	75	47	−78	1.44
Conformer 3	−58	137	78	−143	68	3.91
Conformer 4	−59	145	−64	59	−80	4.24
Conformer 5	−59	143	70	50	68	4.59

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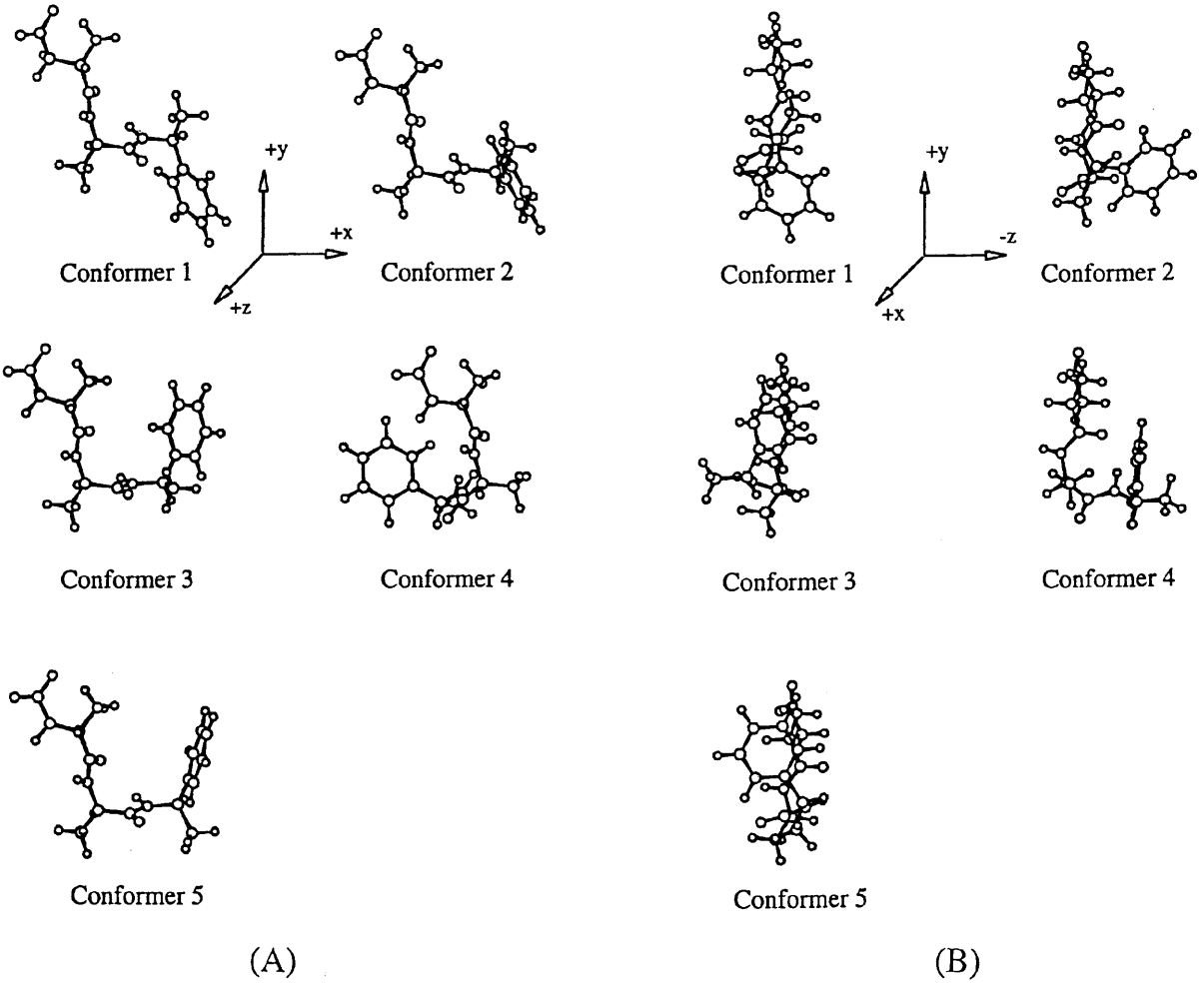

Figure 8 The preferred conformations of compound **III** [Asp-D-Ala-(*R*)-α-MBA] in solution: (A) view 1; (B) view 2.

Table 4 Preferred Conformation of Compound **III** in Solution

	$\chi_1{}^1$	ψ^1	χ^2	ψ^2	ϕ^3	Energy (kcal/mol)
Conformer 1	−59	143	84	−88	79	0.00
Conformer 2	−59	143	83	−85	144	0.79
Conformer 3	−59	143	70	52	74	1.18
Conformer 4	−59	145	−68	70	80	1.94
Conformer 5	−59	139	81	−91	−68	2.67

second residue by substituting the D-aminobutyric acid with α-aminocyclopentane carboxylic acid preserves the sweet taste enhancing conformation. However, this conformation is accompanied by an increased number of other 'non-sweet' conformations with smaller energy differences as compared with compound **I** [Asp-D-Abu-(*S*)-α-EBA], leading to a reduction in sweet taste. The increased number of low energy conformations is a result of the lack of chirality of the second residue.

Eleven low-energy conformations were found for compound **VI** [Asp-Deg-(*S*)-α-EBA]. These conformations can be found in supplementary materials. The torsion angles and the relative energies are listed in Table 7. Compound **VI** showed reduced sweetness potency compared with com-

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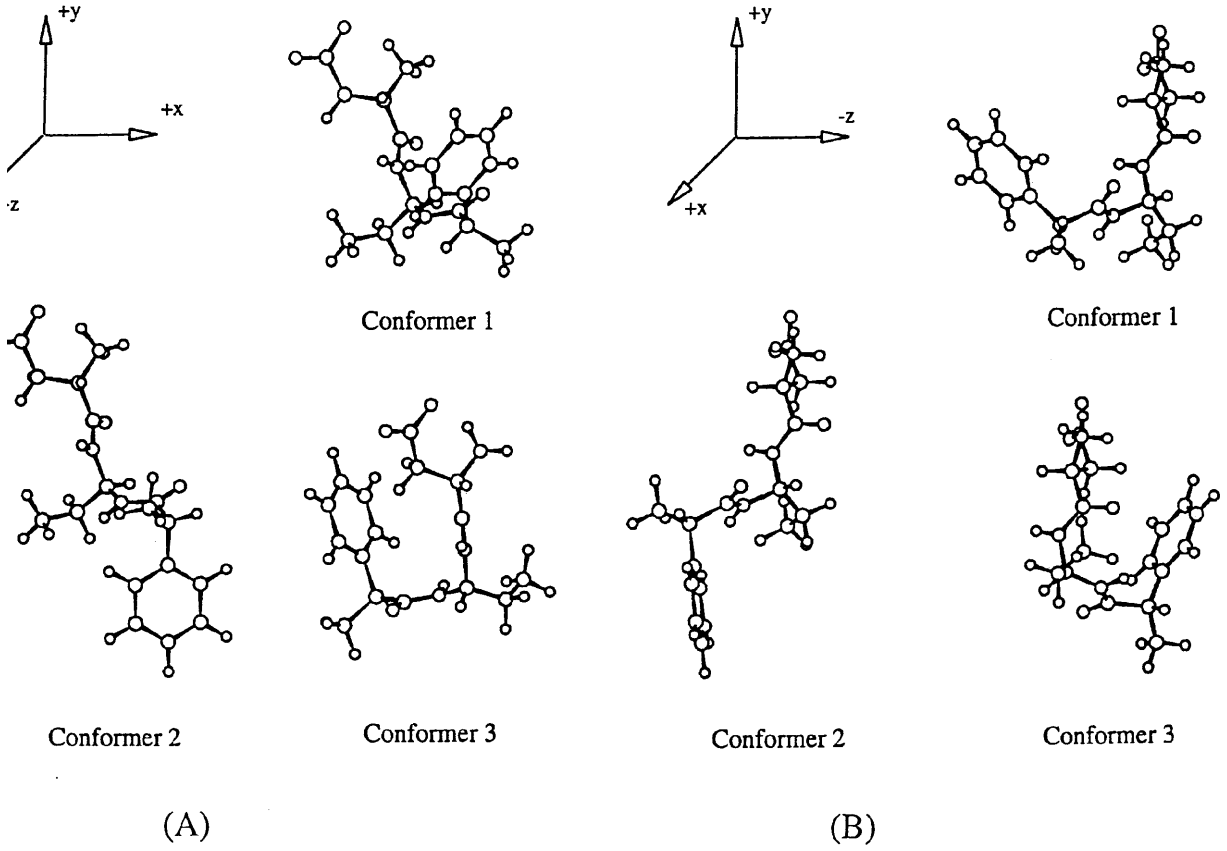

Figure 9 The preferred conformations of compound **IV** [Asp-(*R*)-gAbu-(*S*)-α-PhProp] in solution: (A) view 1; (B) view 2.

Table 5 Preferred Conformation of Compound **IV** in Solution

	$\chi_1{}^1$	ψ^1	χ^2	ψ^2	ϕ^3	Energy (kcal/mol)
Conformer 1	−58	143	144	−92	−98	0.00
Conformer 2	−58	144	148	−99	74	1.50
Conformer 3	−58	137	−46	−76	−88	3.72

Table 6 Preferred Conformation of Compound **V** in Solution

	$\chi_1{}^1$	ψ^1	χ^2	ψ^2	ϕ^3	Energy (kcal/mol)
Conformer 1	−58	136	71	−79	−80	0.00
Conformer 2	−59	146	−73	75	−79	1.45
Conformer 3	−59	146	63	62	−78	1.86
Conformer 4	−59	146	−73	73	−143	2.22
Conformer 5	−59	147	−56	−52	−78	2.28
Conformer 6	−59	145	62	60	−140	2.78
Conformer 7	−58	143	−71	82	73	3.06
Conformer 8	−58	141	73	−74	69	4.03
Conformer 9	−59	140	57	59	69	4.05

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Table 7 Preferred Conformation of Compound **VI** in Solution

	χ_1^1	ψ^1	χ^2	ψ^2	ϕ^3	Energy (kcal/mol)
Conformer 1	−58	136	63	−63	−82	0.00
Conformer 2	−59	145	−43	−47	−83	1.11
Conformer 3	−59	138	62	163	−79	1.99
Conformer 4	−59	149	−47	141	−79	2.02
Conformer 5	−59	147	53	40	−80	2.18
Conformer 6	−58	145	177	145	−79	2.37
Conformer 7	−59	149	−46	139	−140	3.08
Conformer 8	−58	143	65	−55	70	3.59
Conformer 9	−59	143	49	51	69	4.36
Conformer 10	−59	148	−46	131	68	4.47
Conformer 11	−59	152	178	−58	−78	4.86

pound **V** [Asp-Ac^{5}c-(*S*)-α-EBA]. Both analogues have achiral second residues and contain side chains of the same size. However, in compound **V**, the two side chains of the second residue are covalently bonded and form a cyclic structure. In compound **VI**, the two side chains are independent and therefore possess freedom of rotation. The increased conformational flexibility results in greater number of low-energy structures in compound **VI** and therefore further reduces the sweetness potency of this molecule.

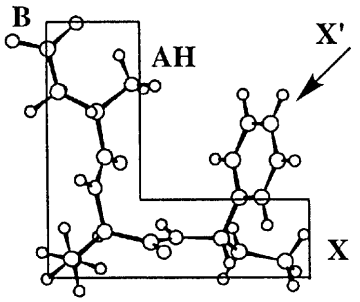

Figure 10 A topological array where the aromatic group X′ is responsible for sweet potency enhancement.

Conclusions

The following is a summary of the observations and conclusions derived from studies of compound **I** and related analogues with both steric and stereochemical variations at the second residue and the C-terminal amide.

First, the correlation of structures and taste properties of the molecules studied support our previous models of molecular basis of taste for dipetide-like sweeteners. Figure 10 shows the 'L'-shape conformation of compound **I** [Asp-D-Abu-(*S*)-α-EBA] which we believe is necessary for the interaction with the dipeptide-like sweetener receptor. In addition to the AH, B and X groups which form the 'L'-shape, we have identified another binding domain above the base of the 'L'. This domain is represented by the phenyl ring in the C-terminus of compound **I** (defined as group X′ in Figure 10). The X′ group is not essential for producing a swee taste but is responsible for enhancement of sweet potency.

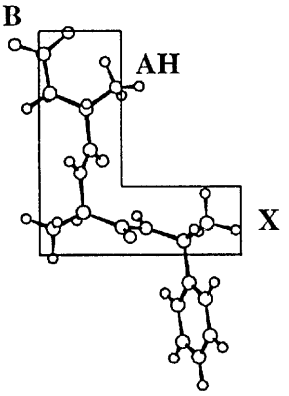

Figure 11 A topological array where the aromatic group of compound **III** [Asp-D-Ala-(*R*)-α-MBA] blocks the interaction between the ligand and sweet taste receptors.

Figure 11 shows the preferred conformation of the non-sweet compound **III** [Asp-D-Ala-(*R*)-α-MBA]. Although the AH, B and X groups form an 'L'-shaped conformation, the displacement of the phenyl ring beneath the base of 'L' blocks the interaction of the

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ligand with the sweetener receptor. As a consequence, this analogue is not sweet.

Second, there has been evidence that the sweetness potency of L-aspartyl-based dipeptide derivatives are dependent on the length and size of the hydrophobic group X. In our model, this corresponds to the length of the base of 'L'. Comparisons of compounds **I** [Asp-D-Abu-(*S*)-α-EBA] and **II** [Asp-D-Abu-(*S*)-α-MBA] suggest that the optimum length of the base of the 'L', measured from the Cα atom of the second residue to the carbon atom of the C-terminal methyl group, is at least 6.33 Å.

Third, according to the model proposed by Ariyoshi, when the C-terminal of a peptide-based ligand functions as the hydrophobic group X, the configuration of the second residue should be D. In our studies, we have shown that molecules with an achiral second residue can also produce a sweet taste but with reduced potency compared with the corresponding analogue with a D residue at the second position. In addition, our studies also showed that a molecule with more constrained side chains at the second residue produces a stronger sweet taste when the second residue is achiral.

Acknowledgements

E.B. gratefully acknowledges MURST, the Ministry of University and of the Scientific and Technological Research, the National Council of Research, CNR and CEINGE, for their continuous and generous support. We would also like to thank the sweetener research group at the Coca-Cola Co., (G. E. DuBois, G. A. Iacobucci, J. G. Sweeny, L. L. D'Angelo, G. A. King III and E. A. Ricks) for samples of compounds **I**–**III**.

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Supplementary Figure 1 The preferred conformations of compound **II** [Asp-D-Abu-(*S*)-α-MBA] in solution: (A) view 1; (B) view 2.
Supplementary Figure 2 The preferred conformations of compound **V** [Asp-Ac^{5}c-(*S*)-α-EBA] in solution: (A) view 1; (B) view 2
Supplementary Figure 3 The preferred conformations of compound **VI** [Asp-Deg-(*S*)-α-EBA] in solution: (A) view 1; (B) view 2

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