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# X-Ray Structures of New Dipeptide Taste Ligands

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Abstract: The molecular basis of sweet taste was investigated by carrying out the crystal state conformational analysis by X-ray diffraction of the following dipeptide taste ligands: *N*-3,3-dimethylbutyl-aspartyl-phenylalanine methyl ester, **I** (*N*-DMB-Asp-Phe-OMe), its sodium salt (*N*-DMB-Asp-Phe-ONa), **II**, aspartyl-D-2-aminobutyric acid-(*S*)-α-ethylbenzylamide, **III** (Asp-D-Abu-(*S*)-α-ethylbenzylamide), aspartyl-*N*′-((2,2,5,5-tetramethylcyclopentanyl)-carbonyl)-(*R*)-1,1-diamino-ethane, **IV** (Asp-(*R*)-gAla-TMCP), and aspartyl-D-valine-(*R*)-α-methoxymethylbenzyl amide, **V** (Asp-D-Val-(*R*)-α-methoxymethylbenzylamide). With the exception of the sodium salt **II**, all compounds are sweet-tasting, showing in some cases considerable potency enhancement with respect to sucrose. The results of this study confirm the earlier model that an 'L-shape' molecular array is essential for eliciting sweet taste for dipeptide-like ligands. In addition, it was established that (i) substitution of the N-terminal group does not inhibit sweet taste, if its zwitterionic character is maintained; (ii) a hydrophobic group located between the stem and the base of the L-shape could be responsible for sweetness potency enhancement, as found in **I, III** and **IV**; in fact, the extraordinary potency of the N-alkylated analogue **I** would support a model with an additional hydrophobic binding domain above the base of the 'L'; (iii) removal of the methyl ester at the C-terminus of compound **I** with the salt formation gives rise to the tasteless compound **II**; (iv) for the first time all possible side-chain conformers ($g^-$, $g^+$ and $t$) for the N-substituted aspartyl residue were observed; and (v) a retro-inverso modification, incorporated at position 2 of the dipeptide chain, confers greater flexibility to the molecule, as demonstrated by the contemporary presence of six conformationally distinct independent molecules in the unit cell and yet sweet taste properties are maintained, as found in **IV**. © 1998 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: Conformational analysis; peptide-based taste ligands; artificial sweeteners; X-ray crystal structures

## INTRODUCTION

The transduction of taste is believed to be initiated by the formation of a host–guest complex between taste ligands and receptor proteins located on the surface of taste cells. Extensive studies have been focused on various ligands in order to probe the molecular basis of taste, since no taste receptors have been isolated to date. Comparisons among a wide variety of sweet compounds revealed 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

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form intermolecular hydrogen bonds with receptor molecules. Further studies have suggested that in addition to the AH and B groups, a hydrophobic region (X) in a ligand is also necessary to elicit sweet taste [2,3].

The discovery of aspartyl-phenylalanine methyl ester (aspartame) [4], a dipeptide with intense sweet taste (approximately 200 times sweeter than sucrose), stimulated extensive structure–activity relationship studies [5]. In Asp-Phe-OMe the protonated amino acid and carboxylate groups of the N-terminal residue aspartic acid represent the AH and B groups of the Shallenberger and Acree model, while the aromatic side chain of the phenylalanine residue functions as the hydrophobic moiety X. Modifications of the structure of aspartame suggested that, in addition to the necessary glucophores, it is also critical for a sweet-tasting ligand to adopt an appropriate three-dimensional structure. Through extensive conformational studies of aspartame and related analogues by X-ray crystallography, $^1$H NMR and molecular mechanics calculations, we have developed a model describing the molecular arrays responsible for sweet and bitter taste [6]. According to our model, the overall topology of sweet-tasting molecules 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. The topochemical arrays accessible to the dipeptide taste ligands can be divided into five categories: I, an 'L-shaped' structure with the AH- and B-containing residue of the N-terminus forming the stem of the L in the $y$-axis and the hydrophobic X group projecting along the base of the L in the $+x$-axis; II, a reversed 'L-shaped' structure with X projecting along the $-x$-axis; III, an extended structure in which the AH/B moiety and the X group form an angle of nearly 180° in a planar linear array, and the X group projects into the $-y$ dimension; IV, a structure with the X group pointing into the $+z$ dimension; V, a structure with significant extension of the X group into the $-z$ dimension.

In the present study, the crystal state structures, derived by X-ray crystallographic analyses, of the following dipeptide-like sweeteners have been determined:

1. *N*-(3,3-dimethylbutyl)-aspartyl-phenylalanine methyl ester (*N*-DMB-Asp-Phe-OMe [7], **I**), which contains an alkyl substitution at the N-terminus with respect to Asp-Phe-OMe and is 7000 times more potent than sucrose (35 times more potent than Asp-Phe-OMe). Two differently solvated, nearly isomorphous crystalline modifications of **I** have been determined, in which the dipeptide taste ligand assumes different conformations in the side chain of the aspartyl moiety.
2. The sodium salt of *N*-(3,3-dimethylbutyl)-aspartyl-phenylalanine (*N*-DMB-Asp-Phe-ONa, **II**), which is a tasteless compound.
3. Aspartyl-D-2-aminobutyric acid-(*S*)-α-ethylbenzylamide, (Asp-D-Abu-(*S*)-α-ethyl-benzylamide, **III**), and aspartyl-D-valine-(*R*)-α-methoxymethylbenzylamide, **V**, which are about 2000 and 1350 times more potent than sucrose [8], respectively, and contain a third chiral centre in the C-terminal ester moiety.
4. Aspartyl-*N*′-((2, 2, 5, 5-tetramethylcyclopentanyl) carbonyl)(*R*)-1,1-diaminoethane (Asp-(*R*)-gAla-TMCP, **IV**) a dipeptide-like sweetener with a retro-inverso modification of the peptide backbone structure. This kind of modification has led to several sweet-tasting compounds [9,10].

The observed crystal state conformations of these molecules are compared with the results of the conformational analysis in solution using high-resolution $^1$H-NMR measurements and computer simulations [11], in an effort to correlate their taste properties and to probe the molecular basis of our taste model. The chemical structures of the molecules investigated are shown in Figure 1.

## MATERIALS AND METHODS

### Materials

Compounds **I** and **II** were synthesized at Bioresearch Inc., San Diego, according to the procedure reported by Nofre and Tinti [7]. Compound **III** was synthesized at the Coca Cola Company [8], compounds **IV** [9] has been previously synthesized in our laboratories, while compound **V** was synthesized at the Ajinomoto Co [11].

### X-ray Diffraction Analysis

Single crystals of the compounds were obtained by slow evaporation of water–methanol or water–isopropanol mixtures. Unit cell parameters were determined by least-squares refinement of the setting angles of a variable number (not less than 20) of high angle reflections ($16° < \theta < 30°$). Unit cell parameters and other relevant crystal data are reported in Table 1. The X-ray data collections of the two crystalline

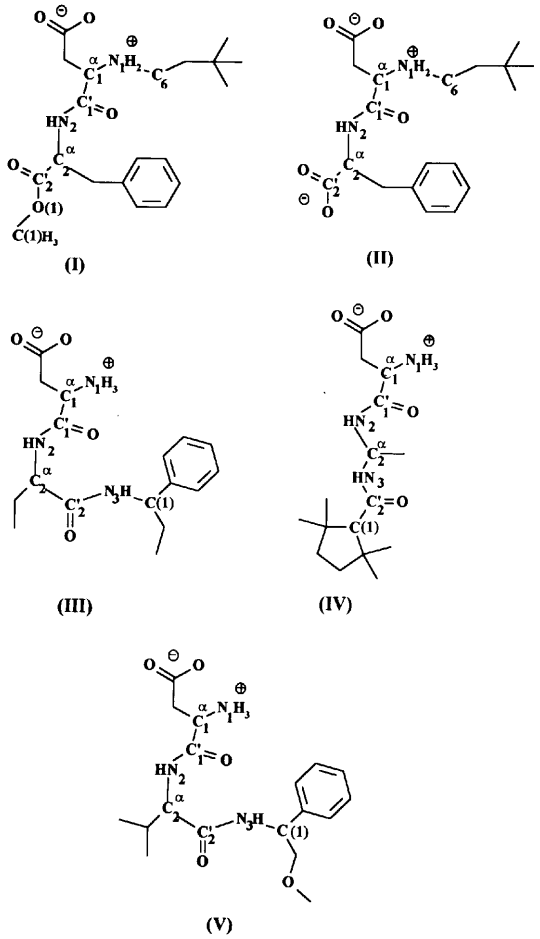


Figure 1 The chemical structures of the compounds investigated in this study.

modifications of **I** were carried out on a Siemens R3m/V diffractometer of the X-ray facilities at the Department of Chemistry & Biochemistry, UCSD, using graphite-monochromated MoK$\alpha$ radiation ($\lambda = 0.71069$ Å). A Rigaku AFC6R diffractometer with graphite-monochromated CuK$\alpha$ radiation ($\lambda = 1.54178$ Å) of the Scripps Research Institute, San Diego, was employed for data collection of **II** and **V**, and an Enraf-Nonius Turbo CAD4 diffractometer of the Biocrystallography Research Center of the CNR at the Chemistry Department of the University of Napoli 'Federico II' was employed for data collection of **III** and **IV**, using graphite-monochromated CuK$\alpha$ radiation ($\lambda = 1.54178$ Å). The structures of **I** in its two crystal modifications (**IA** and **IB**), **II**, **III** and **V** were solved by direct methods using SHELX [12], and the structure of **IV** was solved with an improved version of the SIR 92 program [13]. The best E maps revealed most of the non-H atoms in all structures with a greater difficulty for compound **IV** for which six different molecules are present in the asymmetric unit (154 independent atoms, including solvent molecules). The remaining atoms in each structure and the O atoms of the co-crystallized water molecules were found from subsequent Fourier syntheses. The instability in the air and under the X-ray beam of the crystals of **IB** resulted in a rather poor set of observed reflections, which consequently led to an unsatisfactory refinement of the atomic positions, although the main features of the structure are unquestionable. 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. Refinements of the structures were performed by full matrix least-squares procedures, minimizing the quantity $\Sigma w(F_o - F_c)^2$ using SHELXL 93. For structures **IA, IV** and **V** all non-H atoms were refined anisotropically. For structure **IB** only oxygen atoms that did not show statistical positions were refined anisotropically. For structure **II** oxygen atoms, amide nitrogen, methyl group carbon atoms and the sodium atom were refined anisotropically, and all others were refined isotropically. H atoms were introduced in the calculations with isotropic thermal factors equal to the Beq of the carrier atom and their parameters were not refined. Final $R$ and $R_w$ values together with the reflections used in the refinements and other relevant crystallographic indicators are listed in Table 1. The scattering factors for all atomic species were calculated from Cromer and Waber [14]. Atomic parameters, bond distances and bond angles have been deposited with the Cambridge Crystallographic Data Bank.

## RESULTS AND DISCUSSION

Figure 2 shows the stereo drawings, as determined by X-ray diffraction, of the molecular models of **I**, in its two crystalline modifications (**IA** and **IB**) **II, III** [15], **IV** (**IVA–IVF**) and **V**. The six molecules observed for **IV** are in conformationally similar pairs and therefore only three of them are reported. All molecules crystallize as hydrates: 1 water molecule for **IA**, 2 molecules of water for **II** and **V**, 2.5 molecules of water and 0.5 molecule of methanol for **IB**, 3 molecules of water for **III** and 16 molecules of water for **IV**. As is usually observed in dipeptides of the aspartame family, the N-terminal aspartyl moiety exists as a zwitterion. This is not only observed for **III, IV** and **V** in which an unsubstituted N-terminus is present, but also for **IA, IB** and **II**, in

Table 1 Crystal Data of *N*-DMB-Asp-Phe-OMe (**I**) in its Two Crystalline Forms (**IA** and **IB**), the Sodium Salt *N*-DMB-Asp-Phe-ONa (**II**), Asp-D-Abu-(S)-α-ethylbenzylamide (**III**), Asp-(*R*)-gAla-TMCP (**IV**) and Asp-D-Val-(*R*)-α-methoxymethylbenzylamide (**V**)

| | | **IA** | **IB** | **II** | **III** | **IV** | **V** |
|---|---|---|---|---|---|---|---|
| Empirical formula | | $C_{20}H_{30}N_2O_5$ $H_2O$ | $C_{20}H_{30}N_2O_5$ $2.5H_2O \cdot 1/2CH_3OH$ | $C_{20}H_{29}N_2NaO_5$ $2H_2O$ | $C_{17}H_{25}N_3O_4$ $3H_2O$ | $6[C_{16}H_{29}N_3O_4]$ $16H_2O$ | $C_{18}H_{40}N_3O_5$ $2H_2O$ |
| Formula weight (Da) | | 396.5 | 436.0 | 436.5 | 389.4 | 2252.7 | 414.6 |
| Temperature (K) | | 189 | 188 | 296 | 293 | 293 | 293 |
| Crystal system | | Monoclinic | Monoclinic | Monoclinic | Monoclinic | Monoclinic | Monoclinic |
| Space group | | $P2_1$ | $P2_1$ | $P2_1$ | $P2_1$ | $P2_1$ | $P2_1$ |
| *Z* molecules unit cell | | 2 | 2 | 2 | 2 | 2 | 2 |
| Unit cell dimensions | *a* (Å) | 12.775(3) | 12.51(2) | 10.158(2) | 10.585(9) | 9.981(2) | 12.074(7) |
| | *b* (Å) | 5.590(3) | 5.95(1) | 5.900(1) | 4.820(3) | 44.00(2) | 4.782(5) |
| | *c* (Å) | 15.219(7) | 17.27(3) | 18.323(4) | 21.265(2) | 14.249(7) | 21.057(8) |
| | β (°) | 102.84(3) | 94.7(1) | 92.98(3) | 95.143(4) | 97.66(3) | 101.29(4) |
| Volume ($Å^3$) | | 1059.7(8) | 1282(4) | 1098.3(4) | 1080.5(4) | 6202(4) | 1192(2) |
| Density (calculated) (mg/$m^3$) | | 1.243 | 1.129 | 1.320 | 1.196 | 1.206 | 1.155 |
| Reflections collected | | 2801 | 1952 | 1936 | 2389 | 12543 | 2060 |
| Observed reflections | | 2554 ($I > 2.0\sigma(I)$) | 1085 ($I > 2.0\sigma(I)$) | 1825 ($I > 2.0\sigma(I)$) | 1257 ($I > 2.0\sigma(I)$) | 7457 ($I > 2.0\sigma(I)$) | 1953 ($I > 2.0\sigma(I)$) |
| $R_{int}$ | | 0.0174 | 0.0508 | 0.0340 | 0.0845 | 0.4491 | 0.0299 |
| Data/restraints/parameter | | 2554/1/258 | 1085/1/136 | 1825/1/171 | 1257/4/232 | 11693/913/1364 | 1953/1/254 |
| Final *R* index (obs. refl.) | | 0.036 | 0.130 | 0.082 | 0.081 | 0.101 | 0.076 |
| *R* indices (all data) | | 0.038 | 0.188 | 0.149 | 0.106 | 0.145 | 0.138 |
| *S* | | 1.49 | 2.70 | 1.11 | 1.08 | 1.08 | 0.86 |
| $(\Delta\rho)_{max}(eÅ^{-3})$ | | 0.23 | 0.55 | 0.48 | 0.26 | 0.68 | 0.46 |
| $(\Delta\rho)_{min}(eÅ^{-3})$ | | −0.22 | −0.43 | −0.36 | −0.23 | −0.30 | −0.24 |

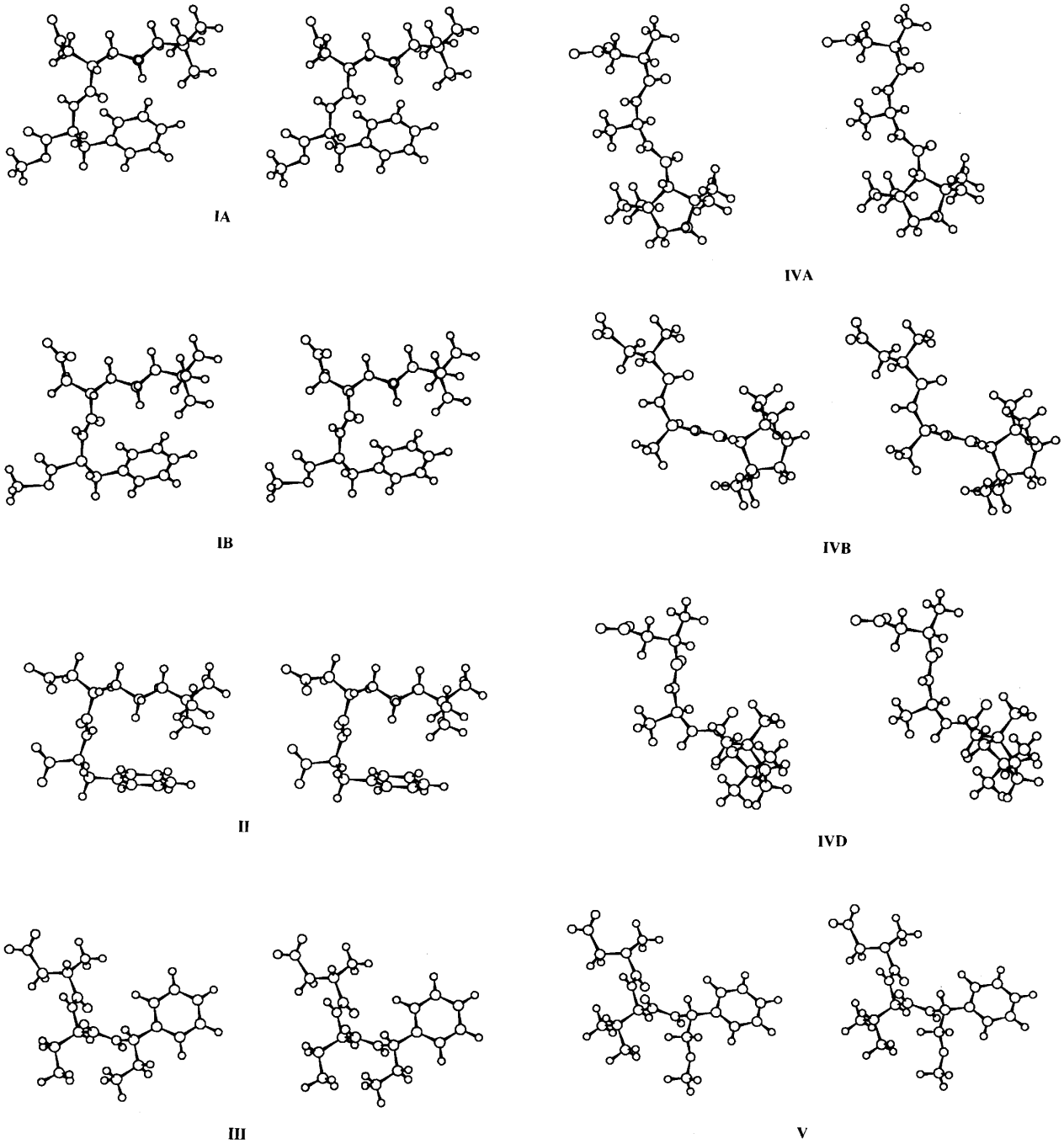


Figure 2 Stereo views of the molecular models of **IA, IB, II, III, IVA, IVB, IVD** and **V** as derived from X-ray diffraction analyses. The molecular models of **IVC, IVE** and **IVF** have been omitted, since they are conformationally closely related to **IVA, IVB** and **IVD**, respectively.

which the N-terminal amino groups have been substituted with the 3,3-dimethylbutyl moiety. In the crystals of **IA, IB, III, IV** and **V** the zitterions of symmetry-related dipeptide molecules face each other. Except for compound **II**, the electrostatic interactions between charged groups are mediated by water molecules, resulting in a rather hydrophilic layer of dipeptide molecules linked to each other and to water molecules with the formation of extensive hydrogen bond networks. In the crystal structure of

the sodium salt **II** one carbonyl oxygen of each carboxylate moiety and one water molecule are involved in the coordination to the metal ion $Na^+$, while the substituted ammonium ion is hydrogen bonded to one carbonyl oxygen of the N-terminal carboxylate group and to the second water molecule.

The existence of two crystal modifications of *N*-(3,3-dimethylbutyl)-Asp-Phe-OMe (**I**) in which the dipeptide assumes different conformations of the Asp side chain moeity ($g^+$ and $g^-$) and the presence of the *trans* conformation for this side chain (*t* conformation) in the crystal structure of **II** prove once more the high flexibility of these taste ligands. A good example for the flexibility of these compounds is found in the structure of **IV**, in which six conformationally different molecules coexist in the crystal.

The conformational angles experimentally found are given in Table 2. Molecules **IB, III, IVB, IVE** and **V** have the L-aspartyl C-terminal residue in the conformation usually observed in aspartyl-based dipeptide analogues [6]. This conformation on average shows dihedral angles $\psi_1$, $\omega_1$, $\chi_1{}^1$ and $\chi_1{}^{2,1}$, of the Asp residue of +156°, +175°, −69° and −173° (or +7° for $\chi_1{}^{2,2}$), respectively. The torsion angle around the Asp $C^\alpha$–$C^\beta$ bond ($\chi_1{}^1$) is *gauche*$^-$ ($g^-$). The carboxylate of the aspartyl side chain is nearly coplanar with the $C^\alpha$–$C^\beta$ bond: the $\chi_1{}^{2,1}$ and $\chi_1{}^{2,2}$ dihedral angles are close to 180° and 0°, respectively. On the contrary, the molecules in the structures of **IA, IVA, IVC, IVD** and **IVF** show for the first time in the solid state the presence of a $g^+$ conformation for the torsion angle round the $C^\alpha$–$C^\beta$ bond $\chi_1{}^1$ (∼ +60°). Finally, in compound **II** we observe for the first time a *trans* conformation for the Asp $\chi_1{}^1$ side chain (−171°).

It is noteworthy that the crystal structures of **IA** and **IB** are practically superimposable (Figure 3) with differences in the torsion angles not greater than 10°, except for the side chains of the Asp moiety which are substantially different. The structures of **IA** and **IB** are very similar to that of Asp-Phe-OMe except for the conformation of the side chain of the C-terminal Phe residue ($\chi_2{}^1$) which is $g^+$ in Asp-Phe-OMe and $g^-$ in **IA** and **IB**. The overall spatial arrangement of the AH, B and X glucophores in **I** is mainly defined by the $\varphi_2$ and $\chi_2{}^1$ torsional angles, since the angles $\psi_1$, $\omega_1$, $\chi_1{}^1$ and $\chi_1{}^{2,1}$ do not substantially change the disposition of the AH and B groups relative to the backbone chain. The conformation observed for compound **I** with values of the $\varphi_2$ and $\chi_2{}^1$ torsional angles of −132° and −62° or −120° and −59° in the structure of **IA** and **IB**, respectively, corresponds to an L-shaped structure in which the AH- and B-containing zwitterionic ring of the aspartyl moiety forms the stem of the L along the +*y*-axis and the hydrophobic X group, represented by the $Phe^2$ side chain, projects along the base of the L along the +*x*-axis. This conformation is typically achieved by compounds in which the hydrophobic element X is represented by the side chain of the L-configurated second residue, as in **I**. It is interesting to note that the conformation of the 3,3-dimethylbutyl substituent does not change both

Table 2 Relevant Torsion Angles for *N*-DMB-Asp-Phe-OMe (**I**) in its Two Crystalline Forms (**IA** and **IB**), the Sodium Salt *N*-DMB-Asp-Phe-ONa (**II**), Asp-D-Abu-(*S*)-α-ethylbenzylamide (**III**), Asp-(*R*)-gAla-TMCP (**IVA–IVF**) and Asp-D-Val-(*R*)-α-methoxymethylbenzylamide (**V**)

| Angle/molecule | | **IA** | **IB** | **II** | **III** | **IVA** | **IVB** | **IVC** | **IVD** | **IVE** | **IVF** | **V** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $C6–N_1–C^\alpha{}_1–C'_1$ | $\varphi_1$ | −78 | −68 | −66 | – | – | – | – | – | – | – | – |
| $N_1–C^\alpha{}_1–C'_1–N_2$ | $\psi_1$ | 174 | 152 | 123 | 145 | −168 | 149 | −171 | 167 | 153 | 163 | 148 |
| $C^\alpha{}_1–C'_1–N_2–C^\alpha{}_2$ | $\omega_1$ | −169 | 180 | 175 | 176 | 175 | 171 | 175 | −175 | 180 | −173 | 176 |
| $N_1–C^\alpha{}_1–C^\beta{}_1–C^\gamma{}_1$ | $\chi_1{}^1$ | 50 | −69 | −171 | −71 | 68 | −70 | 67 | 60 | −63 | 57 | −66 |
| $C^\alpha{}_1–C^\beta{}_1–C^\gamma{}_1–O^{\delta1}$ | $\chi_1{}^{2,1}$ | 41 | −31 | 18 | −11 | −16 | −38 | −15 | −2 | −28 | −11 | −22 |
| $C^\alpha{}_1–C^\beta{}_1–C^\gamma{}_1–O^{\delta2}$ | $\chi_1{}^{2,2}$ | −138 | 153 | −164 | 165 | 164 | 141 | 163 | 172 | 142 | 171 | −156 |
| $C'_1–N_2–C^\alpha{}_2–C'_2$ | $\varphi_2$ | −132 | −120 | −78 | 126 | 119[a] | 78[a] | 122[a] | 138[a] | 75[a] | 141[a] | 127 |
| $N_2–C^\alpha{}_2–C'_2–N_3$ | $\psi_2$ | 148[b] | 151[b] | 160[b] | −117 | −135[c] | −130[c] | −132[c] | −80[c] | −131[c] | −92[c] | −122 |
| $C^\alpha{}_2–C'_2–N_3–C(1)$ | $\omega_2$ | −179[d] | −173[d] | – | 176 | −162[e] | −177[e] | −164[e] | −177[e] | −179[e] | 175[e] | 179 |
| $N_2–C^\alpha{}_2–C^\beta{}_2–C^\gamma{}_2$ | $\chi_2{}^1$ | −62 | −59 | −63 | 177 | – | – | – | – | – | – | 57 |

[a] $C'_1–N_2–C^\alpha{}_2–N_3$.
[b] $N_2–C^\alpha{}_2–C'_2–O(1)$.
[c] This angle for the six molecules of IV is $\varphi'_2$ ($N_2–C^\alpha{}_2–N_3–C'_2$).
[d] $N_3–C'_2–O(1)–C(1)$.
[e] $C^\alpha{}_2–N_3–C'_2–C(1)$.

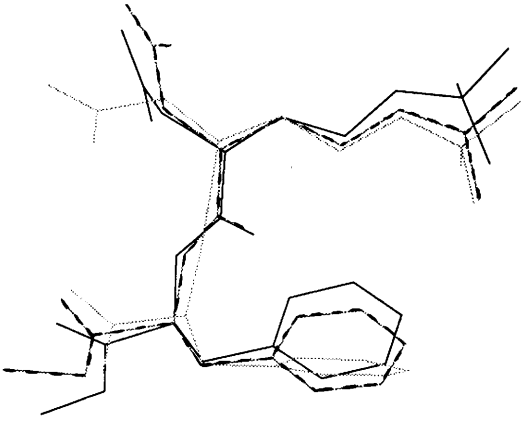

Figure 3 Superposition of the taste ligands of **IA** (bold line), **IB** (thin line) and **II** (dotted line).

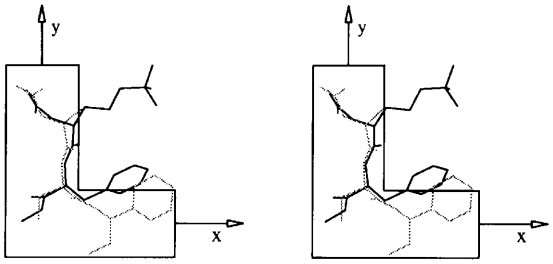


Figure 4 An 'L-shaped' topological array where an additional hydrophobic glucophore could be responsible for sweetness potency enhancement in **I** (solid line) and **III** (dotted line).

the zwitterionic nature of the aspartame-like molecule or the L-shaped structure. Instead the substitution enhances the sweetness potency of this taste ligand, pointing to a possible existence of an additional hydrophobic binding domain above the base of the 'L'. NMR and molecular modelling studies [11] have demonstrated that compound **I** can adopt both extended (class III) and L-shaped (class I) structures with the N-terminal 3,3-dimethylbutyl group running almost parallel to the hydrophobic site X, as observed in the crystal structures of both **IA** and **IB**.

The sodium salt of the *N*-DMB-Asp-Phe-OH ligand **II** shows an unique conformation of the Asp side chain with a *trans* orientation ($\chi_1{}^1 = -171°$) and the carboxylate group almost coplanar with the $C^{\alpha}$–$C^{\beta}$ bond ($\chi_1{}^{2,1} = 18°$ and $\chi_1{}^{2,2} = -164°$ dihedral angles are close to 0° and 180°). However, the rest of the molecule with the $\varphi_2$, $\psi_2$ and $\chi_2{}^1$ torsional angles of −78°, 160° and −63° does not differ substantially from the conformation observed for **IA** and **IB** (see Table 2 and Figure 3 where the superposition of **IA, IB** and **II** is illustrated).

In aspartyl-based dipeptides taste ligands the conformation of the second residue varies greatly, depending upon the configuration and the conformational constraints of the molecules. We have already given a preliminary report on the structure of **III**. [15]. Both molecules **III** and **V** have a second residue with the D-configuration. The D-Abu and D-Val residues adopt in the solid state a semi-extended conformation characterized by dihedral angles $\varphi_2 = 126°$ and $\psi_2 = -117°$ and $\varphi_2 = 127°$ and $\psi_2 = -122°$ in **III** and **V**, respectively. As shown in Figure 4, the bulky C-terminal amide group which represents the hydrophobic group X adopts a topological array 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 or Asp-Val moieties, which extend along the $+y$ direction in **III** and **V**, respectively.

For compound **III**, five conformational families were found in solution [15] with energies not higher than 5 kcal/mol from the lowest energy conformer. Among the preferred conformations of compound **III**, three conformers are characterized by an L-shaped array in which the C-terminal position of the molecule forms the base of the 'L'. The overall topology of the X-ray conformation is very similar to the lowest energy conformation found in solution [15]. In this conformation, the phenyl ring is located above the base of the 'L' and is coplanar with the 'L-shape' array. A comparison of this conformation and the most preferred conformation of the sweet-tasting analogue Asp-Ac$^3$c-O*n*Pr (aspartyl-α-aminocyclopropanecarboxylic acid *n*-propyl ester), which was studied earlier in our laboratories [6], shows that the phenyl ring of compound **III** is not required for sweet taste. However, the intense sweetness of the compound suggests that, as for compound **I**, there might be an additional binding zone in the sweetness receptor which interacts with the phenyl ring located between the stem and the base of the L-shape. This interaction provides great enhancement to the sweetness potency of the compound.

The structure of compound **V** is very similar in conformation and topology to that of compound **III**. For **V** six conformational families were observed in solution [11]. They can be described as L-shaped, extended and semi-extended structures. The conformation observed for **V** in the crystal state is among these solution structures.

It is interesting to note that in molecules **III** and **V** the third chiral centre has an (*S*) configuration with

the C–H bond of the chiral carbon atoms directed out of the average plane of the molecules and parallel to the C=O of the preceding linkage. In this conformation, the two more sterically hindered substituents of the chiral carbon atoms are positioned away from the atoms of the preceding amide bond. The dihedral angles C′2–N3–C3–C4 and C′2–N3–C(1)–C(2) have values of 112.3° and 85° for **III** and **V**, respectively.

We have previously reported the structure of a crystal composed of the two intensely sweet-tasting diastereoisomers Asp-(*R*)-g-Ala-TMCP and Asp-(*S*)-g-Ala-TMCP [16]. In the crystals of **IV** only the Asp-(*R*)-g-Ala-TMCP diastereoisomer is present. In this structure, six independent molecules are found and none of these molecules has a conformation similar to that found previously [16]. The six molecules can be grouped into three conformationally different pairs. One pair has the $\chi_1{}^1$ angle of the Asp side chain in the usual $g^-$ conformation (−70° and −63° for molecules **B** and **E**), as observed previously in the crystal of the diastereoisomeric pair, the other two pairs of conformers have the $\chi_1{}^1$ angle in the $g^+$ conformation (68°, 67°, 60° and 57° for molecules **A, C, D** and **F**, respectively), as observed in the crystal structure of compound **IA**. In all six molecules the carboxylate group is almost coplanar with the $C^\alpha$–$C^\beta$ bond: the $\chi_1{}^{2,1}$ and $\chi_1{}^{2,2}$ dihedral angles are close to 0° and 180°. The $\psi_1$ values for the six molecules are also close to 180° with a mean deviation of 18° and an average value of 169°. However, the molecules with $\chi_1{}^1$ angle in the $g^-$ conformation tend to have $\psi_1$ values of approximately 150° (with a mean deviation of 4° and an average value of 150°), those with $\chi_1{}^1$ in the $g^+$ conformation have $\psi_1$ values closer to 180° (with a mean deviation of 13° and an average value of 177°). The six molecules present again the charged $NH_3{}^+$ and $COO^-$ groups and the amide bond between residue 1 and residue 2 in nearly the same overall topology. The shape of the retro-inverso dipeptide-like molecules is defined by the $\varphi_2$ and $\varphi'_2$ torsion angles: molecules **A** and **C** have on average $\varphi_2$ and $\varphi'_2$ values of 121 and −134; molecules **B** and **E** have on average $\varphi_2$ and $\varphi'_2$ values of 76 and −130, and finally molecules **D** and **F** have on average $\varphi_2$ and $\varphi'_2$ values of 140, −86. These values result in a semi-extended conformation for molecules **A** and **C**, similar to that observed in the three molecules of the crystal structure of alitame [17], in a *quasi* L-shaped conformation for molecules **B** and **E** and in an extended conformation (with extension in the $+z$ direction) for molecules **D** and **F**. These results prove that the retro-inverso modification confers to the molecule a rather high flexibility with the concomitant presence of many conformers in the same unit cell different from that observed in the structure of the diastereoisomeric pair.

Conformational studies by NMR and computer simulations [6,16] have also proved that the molecule can assume at least seven families of different conformations with different topologies of the AH, B and X groups. These topologies vary from extended structures or structures in which the X group extends into the $+z$ dimension or into the space between the $+z$-axis and the $-x$-axis as well as L-shaped or reversed L-shaped structures. However, no structure with significant extension of the X-group into the $-z$ dimension was predicted. Among the different conformers and different topologies observed in the crystal state of the retro-inverso analogue we have never found a conformation with substantial extension of the X group into the $-z$ dimension, which according to our model of sweet and bitter taste gives rise to bitter-tasting compounds [6].

All molecules reported in the present paper as well as most of the sweeteners of the aspartame family crystallize as hydrates with a variable number of water molecules. In the crystal state all hydrogen bond donor groups (the terminal $NH_3{}^+$, $N(R)H_2{}^+$ and the NH amide groups) are involved in a rather complex H-bonding scheme, in which they saturate their capacity of forming H-bonds. In the crystals, electrostatic interactions between zwitterions of symmetry-related molecules are usually modulated by the presence of multiple water molecules which are located in channels between sweetener molecules and participate in the H-bond interactions. The resulting double layers of sweetener and water molecules are held together by hydrophobic interactions.

## CONCLUSIONS

Based upon our studies of dipeptide-like taste ligands with steric and/or stereochemical variations at both the first and the second residue and at the C-terminal amide group the following observations and conclusions can be derived:

1. The correlation of structures and taste properties of the molecules studied supports our previous model for the molecular basis of taste [6]. Figure 4 show the L-shape conformation of compounds **I** and **III** which we believe is responsible for producing sweet taste.

2. Alkylation of the N-terminus with a 3,3-dimethylbutyl group does not alter the zwitterionic character of the dipeptide taste ligand, as observed in compound **I**, which shows enhanced sweetness potency compared with Asp-Phe-OMe.

3. Sweet taste is lost in the sodium salt of N-DMB-Asp-Phe-OH, **II**. The conformation of this ligand does not differ significantly from that of **I**, but the presence of the ionized group at the C-terminus makes it practically tasteless owing to the loss of hydrophobicity.

4. There has been evidence that the sweetness potency of aspartyl-based dipeptide derivatives are dependent on the length and size of the hydrophobic group X. This corresponds to the length of the base of the 'L'. Comparisons of compounds **III** (2000 times more potent than sucrose) and **V** (1350 times more potent than sucrose) with an analogue in which the (*S*)-α-ethylbenzylamide or (*R*)-methoxymethylbenzylamide has been substituted with (*S*)-α-methylbenzylamide (300 times more potent than sucrose) suggest that the optimum length of the base of the 'L', measured from the $C^{\alpha}$ atom of the second residue to the carbon atom of the C-terminal methyl group, is at least 6 Å.

5. According to the model proposed by Ariyoshi, the configuration of the second residue should be D if the C-terminus of a peptide-based ligand functions as the hydrophobic group X. In our studies, we have previously [15] shown that molecules with an achiral second residue can also produce sweet taste but show reduced potency compared with the corresponding analogue with a D residue at the second position. The retro-inverso analogue **IV** with (*R*) chirality at the second residue introduces a substantial modification to the peptide backbone, conferring greater flexibility to the chain. However, this compound is still able to maintain sweet taste properties. Our previous studies [6] in fact have shown that a molecule with more constrained side chains at the second residue produces a stronger sweet taste even if the second residue is achiral.

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