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Molecular basis of sweet taste in dipeptide taste ligands*

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Abstract: In this presentation, we describe an integrated approach for the molecular basis for sweet taste among dipeptide-based ligands. By comparing the results obtained from X-ray diffraction studies with the conformations from NMR analysis and molecular modeling, we have observed recurring topochemical motifs that agree with previous models for sweet taste. In our examination of the unexplored D zone of the Tinti–Nofre model, we have uncovered a sweet potency enhancing effect of a new set of aralkyl-substitutions on dipeptide ligands, which reveals the importance of aromatic–aromatic interactions in maintaining high potency.

INTRODUCTION

In our efforts to determine the molecular basis and structural requirements for the sweet taste, it has been a goal in our laboratories to develop useful molecular models for taste recognition. Our approach toward this end integrates the study of taste profiles with the design, synthesis, and conformational analysis of novel ligands. In constructing predictive taste models, we have sought to determine the active conformations of sweet taste ligands.

Over the years, a number of methods have been applied to examine the conformational preferences of potent sweet taste ligands. Recently, Walters et al. [1] reported the use of computational methods to find conformational similarities within a set of high-potency sweeteners. The resulting topological agreements form a tentative model for sweet taste, although comparisons are made among peptide and nonpeptide structures. This raises the question as to whether all sweet compounds bind to the same receptor with the same conformational interactions. Therefore, we have restricted our studies to peptide-based sweeteners. To obtain active ligand conformations for these interactions, we utilize a combination of NMR spectroscopy, molecular modeling, and X-ray diffraction studies. We believe this combination of biophysical data provides the most effective way to arrive at the topochemical requirements for sweet taste.

Aspartame-based sweet taste ligands

The taste recognition model shown in Fig. 1 describes the relationship between topochemical array and taste in aspartyl-based ligands [2]. The zwitterionic glucophore (denoted AH/B) of the Shallenberger–Kier model is oriented on the +y axis, and the hydrophobic glucophore (denoted X) is allowed to occupy a number of regions in space. The orientation of this hydrophobic group plays a decisive role in determining the taste class of the ligand.

Initial studies aimed at supporting this model focused on a number of aspartame derivatives with substantial potency. Some representative examples are shown in Fig. 2. The retro-inverso amino acid

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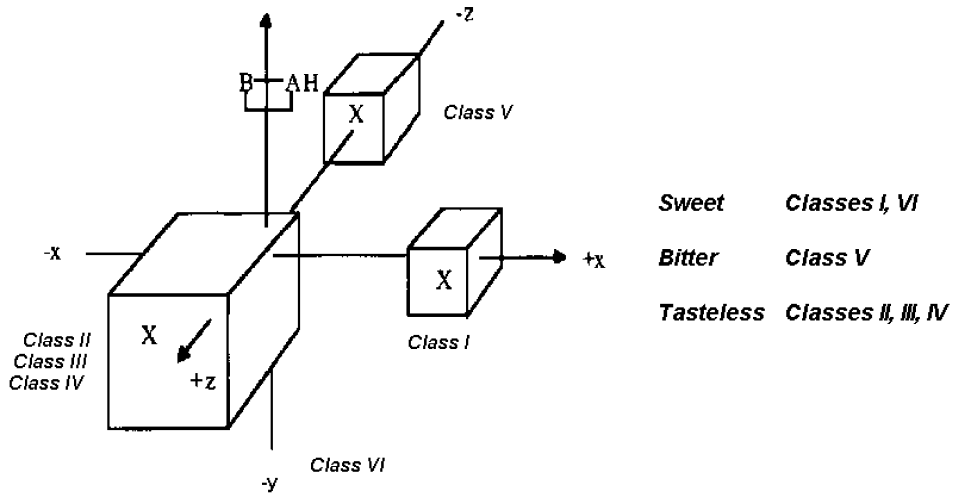

Fig. 1 A schematic illustration of the relationship between topochemichal arrays of the AH, B, and X glucophores and tastes of dipeptide-based ligands [2].

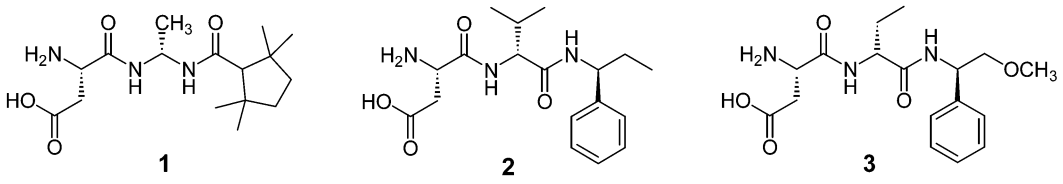

Fig. 2 Aspartame-based sweet taste ligands.

derivative, Asp-(R)-*g*Ala-OTCMP (**1**), exhibits a potency 1200 times that of sucrose. The remaining two structures represent substituted dipeptide benzyl amides (**2** and **3**), which are approximately 1300 and 2500 times sucrose potency, respectively [3,4].

Molecular modeling and NMR analysis of Asp-(R)-*g*Ala-OTCMP (**1**) in solution yields six preferred conformations. These are comprised of two main conformational families (Fig. 3 [2]): the L-shaped (2 clusters) and the extended conformations (4 clusters). From the standpoint of the taste recognition model in Fig. 1, the hydrophobic glucophore of the L-shaped occupies the +x axis region of space, and that of the extended form lies along the –y axis. Both of these conformers contribute to the sweet taste of this ligand.

Studies by X-ray diffraction reveal six independent crystal structures in the unit cell for compound (**1**) [5]. As with the solution-based conformations, each of these crystal structures belongs to either the L-shaped (4 structures) or the extended family (2 structures) as shown in Fig. 4. Comparison of the extended form crystal structure with the representative NMR-based extended conformer shows essentially superposable structures. The same holds true for comparison between the L-shaped crystal structure and the L-shaped NMR-based conformers. In the case of Asp-(R)-*g*Ala-OTCMP (**1**), there is a significant agreement between X-ray and NMR-based data.

Recurring similarities between crystal structures and solution-based conformers prompted us to explore both the L-shaped and extended conformer families in-depth. The Tinte–Nofre model for sweet taste ligands, shown in Fig. 5 [6], assigns spatial regions to a number of pharmacophoric groups considered to be essential for sweet taste. The G domain of this model can be viewed as equivalent to the X domain in the Shallenberger–Kier model since it accommodates the hydrophobic group. The D zone

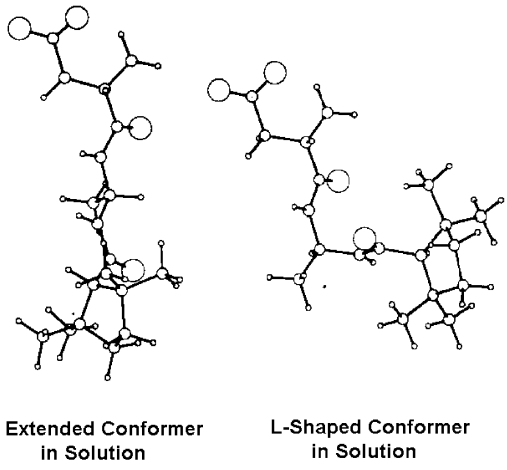

Fig. 3 Conformations of Asp-(R)-*g*Ala-OTCMP (**2**) determined by NMR and molecular modeling.

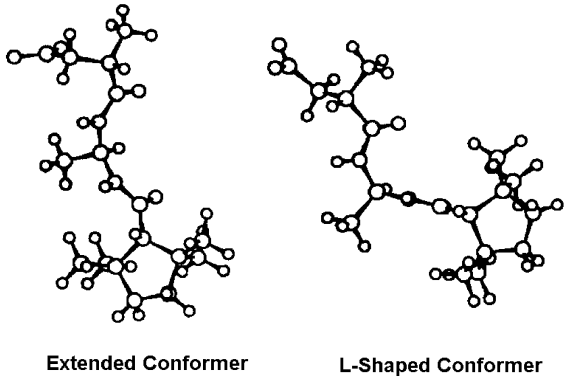

Fig. 4 X-ray structures of Asp-(R)-*g*Ala-OTCMP (**2**).

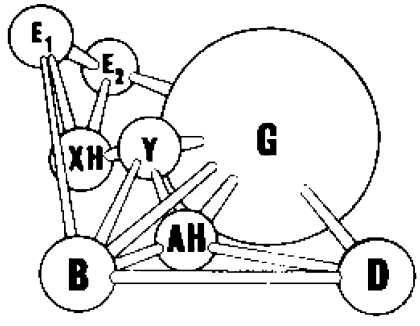

Fig. 5 Tinti–Nofre model for glucophore orientation in dipeptide-based sweet taste ligands [5].

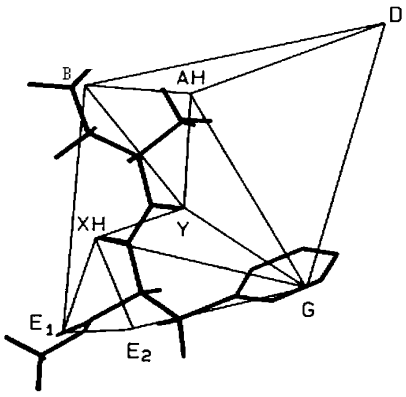

Fig. 6 Superposition of the Tinti–Nofre with L-shaped aspartame [2].

is an intriguing region because we believe it to be an essential spatial component that enhances the potency of sweet ligands.

By placing the Tinti–Nofre model on the Cartesian coordinates and arraying an L-shape aspartame conformer, it is easily seen that the G region of the Tinti–Nofre model superposes on the X region of the Shallenberger–Kier model. The D zone remains unexplored in terms of molecular arrangement (Fig. 6 [2]). As a result, we sought to design and synthesize sweet ligands to probe this D zone and to determine its role in sweet taste potency.

N-Dimethylbutyl-substituted sweet taste ligands

Figure 7 shows three dipeptides, each bearing an *N*-3,3-dimethylbutyl group (DMB). The most potent compound, neotame (**4**), is DMB-substituted aspartame and is 7000 times sweeter than sucrose. The α-methyl phenylalanine analog of neotame (**5**) is slightly less potent than its parent compound, and the dipeptide *N*-DMB-Asp-(D)Val-(S)-α-ethylbenzylamide (**6**) is the least potent of the group at 3000 times the sweetness of sucrose [4,7].

When examining the preferred conformations in solution as determined by NMR and molecular modeling, each compound exhibits both extended and L-shaped clusters. Each of these conformations shows the dimethylbutyl group probing the D zone. Among all of the accessible topologies in solution, only the reversed-L shape does not explore this D zone. The preferred conformations in solution of neotame are shown as representative examples (Fig. 8 [4]). In the case of the least potent analog, the D zone is not as fully occupied as in neotame or α-MePhe neotame. From this, we deduced that the occupancy of the D zone in fact plays a role in enhancing the potency of peptide-based sweeteners.

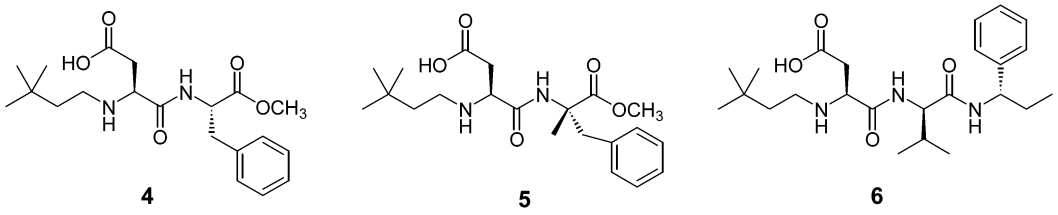

Fig. 7 Structures of *N*-3,3-dimethylbutyl substituted sweeteners.

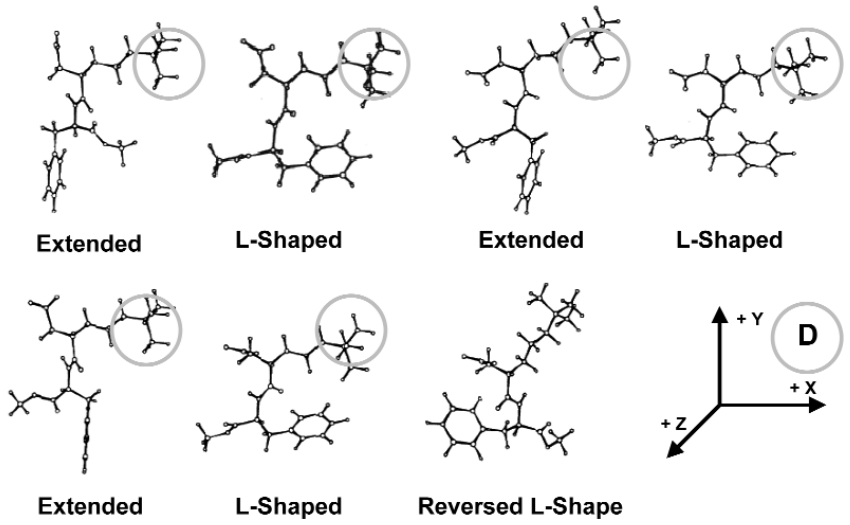

Fig. 8 Preferred conformations in solution of compound neotame (**4**) determined by NMR and molecular modeling. The D zone is circled for each structure.

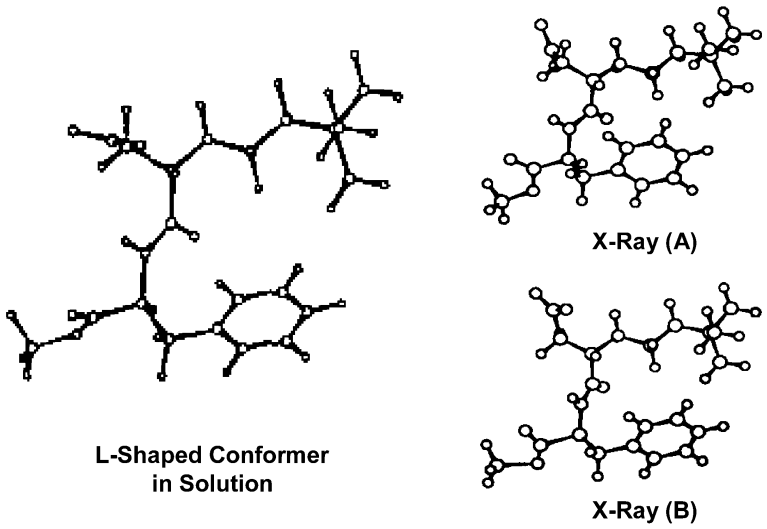

Fig. 9 Comparison of X-ray structures to the L-shaped conformer in solution of neotame (**4**).

The X-ray diffraction structure of neotame (**4**) indicated a monoclinic structure with two molecules in the unit cell [2]. Both structures are L-shaped and array the DMB groups in the D zone. A comparison of the X-ray structures with the L-shaped conformers we obtained from NMR and molecular modeling shows striking similarities (Fig. 9).

Sweet taste ligands with *N*-aralkyl substitution

At this point, we turned to novel *N*-substituted structures bearing aromatic groups on the aliphatic chain (Fig. 10). The dimethyl-methoxyphenol analog (**7**) exhibits a potency of about 50 000 times that of

sucrose. The methoxyphenol compound without the methyl groups (**8**) is approximately half as sweet (25 000 times sweeter than sucrose). The dimethylphenyl (**9**) analog is 4000 times sucrose potency, and the 3-phenyl propyl analog (**10**) is only 1000 times sweeter than sucrose.

For these analogs, the preferred conformations in solution (determined by NMR and molecular modeling) include both the L-shaped and the extended conformations. In terms of the topochemical analysis, these two families appear to be the most important. Relative energy values for the preferred conformations show that the most stable of these is the L-shaped for each analog. This is most pronounced in the case of the dimethyl-methoxyphenol analog (**7**) [8].

The extended forms (Fig. 11) of compounds **7–10** show that each analog assumes essentially the same topology. There appears to be no discernible trend that correlates with sweet potency. However, with the L-shaped conformers shown in Fig. 12, a significant trend is evident. There is a clear interaction between the methoxyphenol group of compound **7** and the aryl group at the base of the L-shaped stem. The same is true with the methoxyphenol (**8**) analog. In the case of the other analogs, a diminution of the aromatic–aromatic interactions is concominant with a reduction in sweet taste potency.

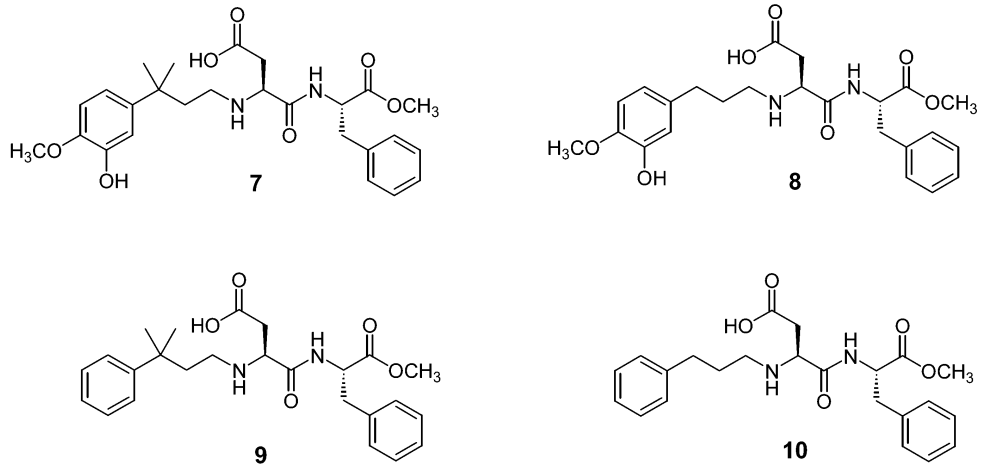

Fig. 10 Sweet taste ligands with *N*-aralkyl substitution.

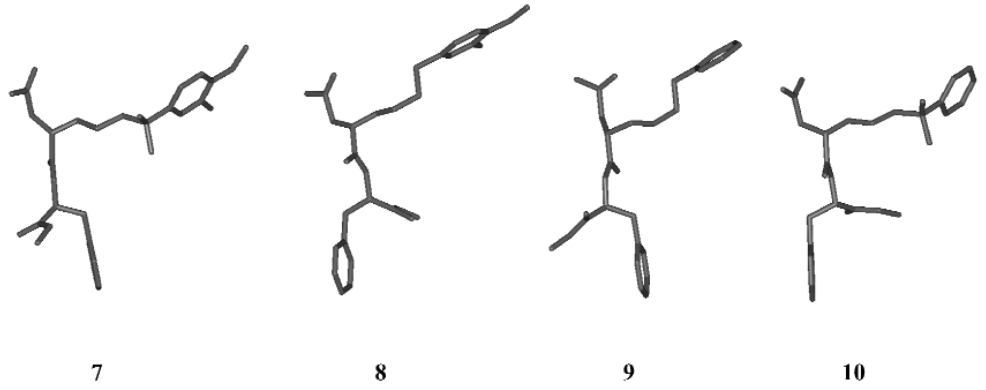

Fig. 11 Extended conformers in solution of compounds **7–10.**

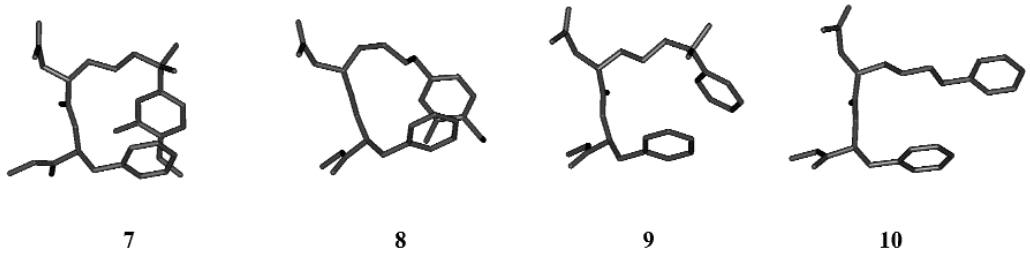

Fig. 12 L-shaped conformers in solution of compounds **7–10**.

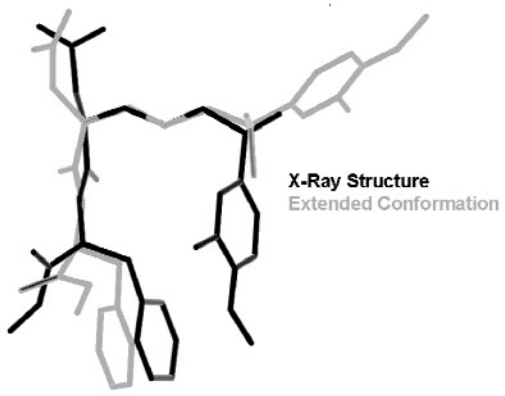

Fig. 13 Superposition of X-ray structure and extended conformer of compound **7**.

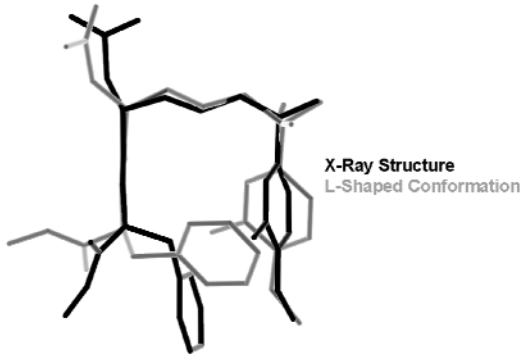

Fig. 14 Superposition of X-ray structure and L-shaped conformer of compound **7**.

Successful crystallization of the dimethyl-methoxyphenol analog (**7**) revealed orthorhomic structure [9]. The superposition of the crystal structure and extended conformer in solution of the dimethyl-methoxyphenol analog (**7**) shows a reasonable correlation for parts of the molecules (Fig. 13). The critical difference, however, involves the distinct spatial arrangements of the dimethyl-methoxyphenol groups. When comparing the X-ray structure to the L-shaped conformer in solution (Fig. 14), both molecules show the same aromatic–aromatic interaction. The small difference in the placement of the aryl

group, attributable to the crystal packing, can be corrected by minor perturbations to the $\chi 1$ and ψ torsion angles of the second residue.

CONCLUSIONS

Based on our observations, we propose that the structures of the aralkyl groups in the D zone are the key to the major enhancement of the sweet potency of compounds **7** and **8**, as compared with a taste ligand such as neotame.

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REFERENCES

1. D. E. Walters, I. Prakash, N. Desai. *J. Med. Chem.* **43**, 1242–1245 (2000).
2. T. Yamazaki, E. Benedetti, D. Kent, M. Goodman. *Angew. Chem., Int. Ed. Engl.* **33**, 1437–1451 (1994).
3. M. Goodman, Q. Zhu, D. Kent, Y. Amino, R. Iacovino, E. Benedetti, A. Santini. *J. Pept. Sci.* **3**, 231–241 (1997).
4. R.-H. Mattern, Y. Amino, E. Benedetti, M. Goodman. *J. Pept. Res.* **50**, 286–299 (1997).
5. M. Goodman, R.-H. Mattern, P. Gantzel, A. Santini, R. Iacovino, M. Saviano, E. Benedetti. *J. Pept. Sci.* **4**, 229–238 (1998).
6. J. M. Tinti and C. Nofre. In *Sweeteners: Discovery, Molecular Design, and Chemoreception,* D. E. Walters, F. T. Orthoefer, G. E. Dubois (Eds.), American Chemical Society, Washington, DC (1990).
7. R.-H. Mattern, Y. Amino, E. Benedetti, M. Goodman. *Biopolymers* **49**, 523–539 (1998).
8. Details of the design, synthesis, and conformational analysis of these compounds will be reported in a separate paper.
9. Data for the X-ray diffraction analyses will be published in due course.