A three-dimensional receptor model for isovanillic sweet derivatives

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Using pseudoreceptor modelling, we have derived a three-dimensional binding-site model for the structurally uncharacterised sweet-taste receptor. The receptor model was derived based on 17 sweet compounds of the isovanillyl class (4-methoxy-3-hydroxybenzyl) as the training set and consists of nine key amino-acid residues embedded in a hydrophobic receptor cavity. The underlying technology (software PrGen) allows for a dynamical treatment of the ligand–receptor complex (ligand equilibration and Monte-Carlo scanning of receptor space) as well as for receptor-mediated ligand alignment. Free energies of ligand binding are estimated based on ligand–receptor interactions, ligand desolvation energy, change of ligand internal energy and change of ligand entropy upon receptor binding.

The validity of the receptor model has been assessed by using a test set of eight isovanillyl sweet compounds different from the training set. For these ligands, the obtained binding-site surrogate is capable of predicting free energies of ligand binding, ΔG°, to within 0.99 kcal mol^{-1} (rms) of their experimental value, corresponding to an uncertainty in the sweetness of a factor of 5.5. Maximal individual errors of predicted sweetnesses do not exceed a factor of 18.

Introduction

Computational studies on molecular recognition between proteins and low molecular weight molecules have experienced a significant development in the recent decade. Various enzyme and receptor systems of chemical, biological and medicinal interest have been studied by simulating ligand–protein interactions at the molecular level. The mechanism of action of sweet substances, as well as that of other taste compounds, has been under investigation for some time.[1] For these compounds, the existence of a corresponding receptor has still not been confirmed. Several attempts to isolate and characterise the putative peptidic receptor from lingual tissues have failed and, until now, no genetic studies have been published on this topic. Nonetheless, the existence of such a receptor, probably a transmembrane protein coupled to a G-protein, is strongly suggested by several studies.[2] Evidence for this hypothesis is mainly based on neurophysiological and animal behavioural experiments. One uncertainty concerns the existence of a single protein—in contrast to a multiple receptor protein—or, alternatively, a single protein with multiple binding sites. Unfortunately, the various studies have not yet clarified this point.

One of the peculiar characteristics of the sweet taste receptor system is its ability to recognise molecules belonging to very different classes of compounds. Moreover, the behaviour of the sweet-taste receptor towards its substrates can be somewhat considered an anomaly: it is characterised by a very low affinity towards the natural agonists, but at the same time it shows high specificity requirements. For these reasons, one of the current approaches in these studies is the use of models, that have been developed to describe the nature and the topological arrangement of glucophores of an ideal sweet compound or the recognition sites of the sweet-taste receptor.

A first intuitive model was proposed as early as 1967 by Shallenberger and Acree.[3] They recognised the existence of two functional groups, 'glucophores', (referred to as 'AH' and 'B', corresponding to a H-bond donor and a H-bond acceptor, respectively) in almost every sweet molecule. The role of these glucophores in molecular recognition was to engage in two antiparallel hydrogen bonds with complementary sites on the receptor protein (Fig. 1). Kier[4] suggested a third interaction site (originally referred to as 'X'), corresponding to a hydrophobic part of the molecule (Fig. 2). This simple model found widespread acceptance,[5] due to its ability to explain the sweetness of many structurally different compounds and to interpret changes in sweetness due to geometrical or conformational differences, especially in sugars. It has been also of some heuristic value, contributing to the preparation of new sweet substances.

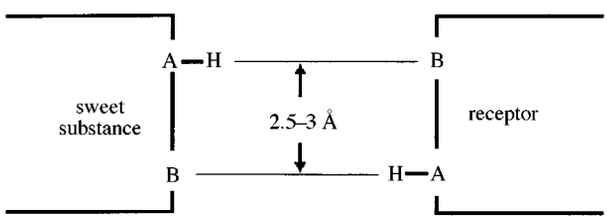


Fig. 1 The Shallenberger–Acree model of a sweet-taste receptor

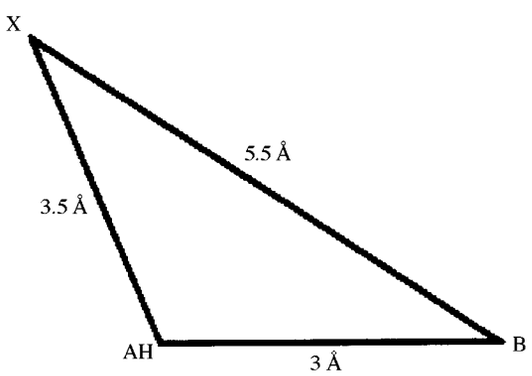


Fig. 2 The Kier model of sweet taste glucophores

Substantial progress in the understanding of the molecular properties of organic compounds and in molecular modelling has provided tools leading to a more elaborate model than the one of Shallenberger and Acree. The first step in this direction was made by Temussi and co-workers[6] who, on the basis of an extensive conformational study of aspartame with nuclear

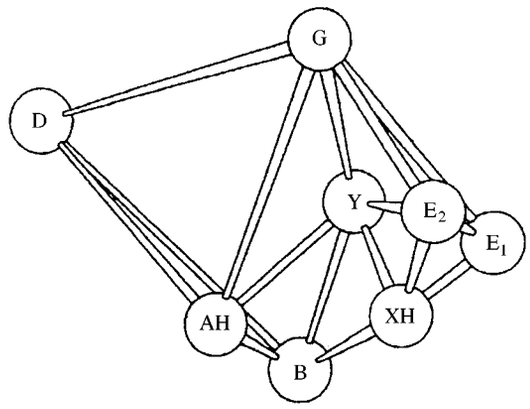


Fig. 3 The 'multi-point attachment' Nofre–Tinti model

magnetic resonance techniques (NMR) and theoretical studies, proposed a model for the sweet receptor. This model depicts the receptor as a hemihedral cavity of defined shape which includes the AH–B groups of Shallenberger and Acree. Moreover, it can explain the change from sweet to bitter of the taste of some enantiomeric compounds, such as some D- and L-amino acids. Modifications to this model were made by Goodman and co-workers.[7] Further progress was made when Nofre and Tinti[8] were able to synthesise new compounds with outstanding sweetness potency, up to 200 000 times that of sucrose, now known as hyperpotent guanidinic sweeteners. Comparison of molecular properties of these extremely sweet substances within a structure–activity relationship study of various sweeteners in man, led to an 'eight-interaction site model', based on the chemical nature and topology of an ideal sweet compound (Fig. 3). This is at present the most detailed model and as such has been successfully used to explain the sweet taste of many compounds belonging to different classes.[7,9–11] In a later work[12] Nofre and Tinti proposed an improved model where they assigned eight amino acid residues of the sweet-taste receptor involved in a total of 15 interactions with the glucophores. Unfortunately, they neither describe which method was used to construct the receptor model nor is the three-dimensional arrangement of the amino acids within this surrogate discussed. Moreover, this model—as well as the previous ones—does not provide any quantitative information about the affinity (sweetness) of the examined compounds towards the receptor model. A peptidic receptor model consisting of an α-helix moiety has been proposed by Suami and Hough,[13] on the basis of the chirality of several sweet compounds.

Some hyperpotent guanidinic derivatives have also been used to raise monoclonal antibodies which have been fully characterised together with some complexes between the hapten and the immunoglobulin.[14] These antibodies could be used as models of the guanidinic sweeteners receptor, but no information about the ability to recognise other sweet substances is yet available.

The study of isovanillic derivatives, a class of compounds structurally related to the natural compound (+)-(*R*)-phyllodulcin[15] and to the semisynthetic sweetener neohesperidin dihydrochalcone (NHDC),[16] has been a tradition at our laboratory. In the last few years, we have synthesised and 'tasted' more than 100 isovanillic derivatives, investigating structure–taste relationships in this class of compounds. Particular attention has been devoted to the role of heteroatoms[17] in the possible interaction with the receptor and to the study of the active conformations.[18] Recently, our interest has been focused on the relationship between taste and configuration: in fact, it is known from the literature that only the (+)-(*R*) enantiomer of phyllodulcin is sweet, while the other is tasteless.[19]

To derive a semi-quantitative structure–activity relationship, we made use of pseudoreceptor modelling. This technique bridges structure-based receptor fitting and property-based receptor mapping, where a paucity of information concerning receptor structures has spawned techniques that project the properties of a set of bioactive ligands into three dimensions around their appropriately superimposed molecular framework. The resulting map provides steric, electrostatic and lipophilic profiles used to identify type and approximate position of receptor residues (or their functional groups) interacting with the ligand at the true biological receptor. Pseudoreceptor modelling allows the construction of a three-dimensional model of the binding site of a structurally uncharacterised bioregulator based on the structures of known ligand molecules.[20] The philosophy underpinning the pseudoreceptor concept is to engage the bound species in sufficient, specific non-covalent binding so as to mimic the essential ligand–macromolecule interactions at the true biological receptor.[21] This approach (software PrGen[22]) would seem to be ideally suited for the sweet-taste receptor system, as nothing is known about its primary, secondary and tertiary structure. This paper reports the construction and validation of a three-dimensional pseudoreceptor for isovanillic sweet compounds.

Experimental

Synthesis of compounds

Literature references for the synthesis and/or taste of compounds are given, as far as available, in Fig. 4. Compound **25** was synthesised by acetalisation of the corresponding aldehyde with 2-mercaptobenzyl alcohol: mp 187 °C, $\delta_H(CDCl_3)$ 4.95 (1H, br, OH), 5.13 (2H, s, H-4), 6.13 (1H, s, H-2), 6.8–7.5 (8H, m, arom.); *m*/*z*(%) 244 (37, M^+), 215 (5), 211 (14), 122 (74), 121 (100).

Tasting

Most of the compounds studied in this work were tasted at our laboratory. A solution of exactly known concentration of about 2% of the compound in absolute ethanol was made and diluted to the desired concentration with freshly distilled water. An untrained panel of 5–7 people tasted the solutions in comparison with 3% sucrose in water, containing the same amount of ethanol, to assess the sweet-taste potency. If a compound was judged sweeter than the standard, it was diluted until an isosweet solution was obtained. The relative sweetness, RS, is defined as $RS = [sucrose]/[sweetener]_{isosweet}$.

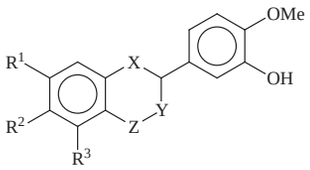
Selection of compounds

Fig. 4 shows the sweet compounds considered in this study along with their relative sweetness and references (where available) on their synthesis and taste. They were selected on the basis of structural similarity and in the widest possible range of biological activity. Most of the compounds have a stereogenic centre and therefore the two enantiomers will have diastereoisomeric interactions with the receptor. All ligand molecules, except **1**, **18** and **22**, which were single enantiomers, were synthesised and subsequently tasted as racemic mixtures. However, as (+)-(*R*)-phyllodulcin **1** is sweet, whereas (−)-(*S*)-**1** is tasteless and a similar behaviour appears from preliminary experiments to hold also for at least two other compounds of the series, the configuration corresponding to that of (+)-(*R*)-**1** was chosen for all the compounds in the modelling procedure.

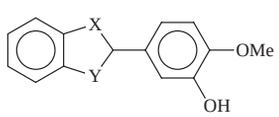
Molecular modelling and pseudoreceptor construction †

The three-dimensional structures of the sweet compounds were built with the software INSIGHT II, 2.3.5 (Biosym Technologies, San Diego, CA) running on a Silicon Graphics

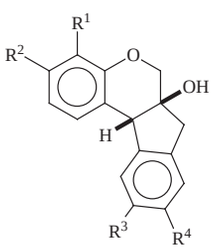
† PDB coordinates of the pseudo-receptor model containing the training set and (separately) the test set coordinates are available as supplementary material (SUPPL. NO. 57378, 8 pp.). The three-dimensional coordinates of ligands and receptor are available for distribution (DISMA@imiucca.csi.unimi.it). Ordering information is given on any current masthead page.



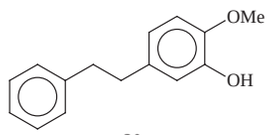
	R^1	R^2	R^3	X	Y	Z	RS	Ref.
1	H	H	OH	CH_2	O	CO	400	15
2	H	H	H	S	O	CH_2	9000	17
3	H	H	H	S	S	CH_2	10000	17
4	H	H	H	O	O	CH_2	3000	31
5	H	H	H	S	CH_2	CH_2	200	17
6	H	H	H	O	S	CH_2	500	17
7	H	H	H	O	CH_2	CH_2	350	18
8	H	H	H	CH_2	O	CH_2	350	18
9	H	H	H	CH_2	CH_2	O	200	18
10	H	H	H	O	CH_2	O	450	18
11	H	H	H	O	CH_2	S	250	17
12	H	H	H	S	CH_2	S	500	17
13	H	OH	H	O	CHOAc	CO	500	17
14	OH	H	OH				500	32



	X	Y	RS	Ref.
15	S	O	300	17
16	O	O	150	18
17	S	S	200	17



	R^1	R^2	R^3	R^4	RS	Ref.
18	OH	OH	OH	OH	120	9
19	H	H	OH	OMe	50	9



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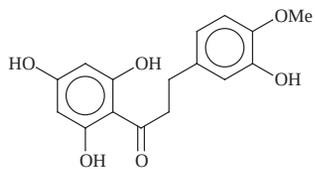

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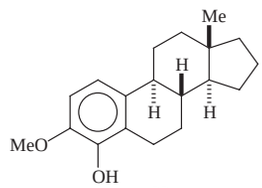

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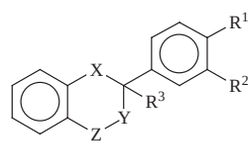
	RS	Ref.
20	300	32
21	550	33
22	50	9



	R^1	R^2	R^3	X	Y	Z	RS	Ref.
23	OMe	OH	H	S	CH_2	O	2000	34
24	OMe	SH	H	S	O	CH_2	50	17
25	H	OH	H	S	O	CH_2	120	10

Fig. 4 Molecular structure, relative sweetness (RS) and literature references of compounds **1**–**25**

IRIS 4D-35GT computer. All ligand molecules were optimised in aqueous solution using the MM2 force field[23] as implemented in the program MacroModel 5.0.[24] This approach uses a continuum-model representation of the solvent instead of discrete (real) water molecules. The molecules were first minimised using the default MacroModel 5.0 charges; then the atomic partial charge model, based on MNDO electrostatic potential charges, was derived using MOPAC 6.0.[25] Another round of minimisation was performed using these final charges. Free energies of ligand solvation were calculated with MacroModel 5.0 and experimental dissociation constants were taken from ref. 26.

Experimental free energies of ligand binding were calculated according to eqn. (1), where $K_d = K_d$(sucrose)/RS and RS = relative sweetness.‡ Throughout this work, the RS values are referred to the racemates, except for **18** and **22**. In PrGen,[20,22] free energies of ligand binding, ΔG°, are estimated based on an approach of Blaney *et al.*[27] [eqn. (2)]. Ligand solvation energies are calculated using the method of Still *et al.*;[26] the loss of entropy upon receptor binding is assessed following Searle and Williams.[28] The fourth term corrects for the deviation of the ligand internal energy (while bound to the pseudoreceptor) from a strain-free reference conformation. To determine the ligand–receptor interaction energy, $E_{\text{ligand–receptor}}$, the program uses a directional force field.[29] Free energies of ligand binding, $\Delta G^\circ_{\text{pred}}$, are then obtained by means of a linear regression (slope a, intercept b) between $\Delta G^\circ_{\text{exp}}$ and E_{binding} using the ligand molecules of the training set [eqn. (3)].

$$\Delta G^\circ = RT \times \ln K_d \quad (1)$$

$$E_{\text{binding}} \approx E_{\text{ligand–receptor}} - T\Delta S_{\text{binding}} - \Delta G_{\text{solvation,ligand}} + \Delta E_{\text{internal,ligand}} \quad (2)$$

$$\Delta G^\circ_{\text{pred}} = |a|\, E_{\text{binding}} + b \quad (3)$$

Results and discussion

The first step in pseudoreceptor building, the superimposition of the ligands, is also one of the more critical. To circumvent

‡ Relative sweetness (RS) values have been converted to K_d values. For the mapping process only relative K_d values are relevant (as the predicted values are obtained by a regression, the position on the absolute scale does not matter). As the value of K_d sucrose is unknown, it was arbitrarily set at 10^{-6} M in order to allow for a reasonable range of values for the K_d of the examined compounds.

Table 1 Comparison of experimental and predicted dissociation constants for the sweet-taste receptor surrogate

Sweet compound	Experimental RS[a] (as $\Delta G°$, kcal mol^{-1}[b])	Predicted RS (as $\Delta G°$, kcal mol^{-1}[c])	Error in RS (as $\Delta G°$, kcal mol^{-1})
A (training set)			
3	−13.40	−13.31	0.09
23	−12.47	−11.87	0.60
6	−11.66	−11.78	−0.12
12	−11.66	−11.89	−0.23
13	−11.66	−11.62	0.04
14	−11.66	−11.75	−0.09
1	−11.53	−11.56	−0.03
7	−11.45	−11.43	0.02
8	−11.45	−11.43	0.02
15	−11.36	−11.43	−0.07
20	−11.36	−11.36	0.00
9	−11.13	−11.14	−0.01
17	−11.13	−11.10	0.03
18	−10.83	−11.00	−0.17
25	−10.83	−11.26	−0.43
22	−10.32	−10.24	0.08
24	−10.32	−10.08	0.24
Max. ind. error[d]			0.60
B (test set)[e]			
2	−13.35	−12.23	1.12
4	−12.70	−11.69	1.01
21	−11.72	−11.11	0.61
10	−11.60	−10.99	0.61
11	−11.26	−11.53	−0.27
5	−11.13	−11.73	−0.60
16	−10.96	−12.65	−1.69
19	−10.32	−11.58	−1.26
Max. ind. error[d]			1.69

[a] RS = relative sweetness. [b] *cf.* Text. [c] Correlation coefficient (training set: 17 compounds) = 0.956; rms deviation = 0.208 kcal mol^{-1} (uncertainty factor in relative sweetness 1.4). [d] Maximal error in the prediction of the relative sweetness of an individual sweet compound. [e] Test set (eight compound): rms deviation 0.994 kcal mol^{-1} (uncertainty factor in relative sweetness 5.5).

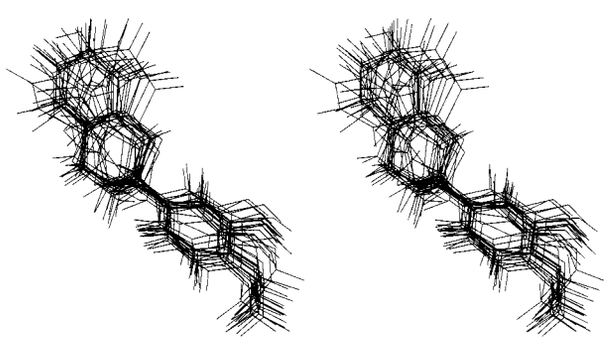


Fig. 5 Overlap of the ligands obtained by receptor-mediated ligand alignment

problems associated with functional group obscuring (by less active compounds), we have applied a technique referred to as *receptor-mediated ligand alignment*. At the beginning only three of the sweetest compounds of the whole dataset, **3**, **23** and **2** were superimposed: since all the compounds contain the isovanillyl ring that is deemed necessary for the elicitation of the sweet taste, we decided to superimpose the aromatic ring of this moiety of the molecules. Three amino acid residues (Arg, His and Ser), interacting with the key functional groups of these molecules, were selected. After full minimisation, the residual 22 compounds were added and allowed to relax within this primordial model. This ligand superposition was treated dynamically (equilibration, Monte-Carlo searches of conformational space) throughout model construction. Free energies of ligand binding were estimated based on ligand–pseudoreceptor interactions, ligand desolvation energy and change of the internal ligand energy and ligand entropy upon receptor binding. This yielded an unbiased ligand superposition with key functional groups of the sweetest compounds not obscured by aliphatic moieties of less sweet compounds, a prerequisite for pseudoreceptor construction. Fig. 5 shows the overlap of the ligands obtained.

Based on this alignment of 25 sweet compounds, to select a training set from the available biological data that span parameter space homogeneously, we made use of a method originally developed by Marengo and Todeschini[30] and adapted for pseudoreceptor modelling by Zbinden and co-workers.[22,§] This allows for an unbiased selection of the most dissimilar molecules from an ensemble of ligands to be used as the training set. Our training set consisted of 17 compounds. At this point the three amino acid residues of the primordial receptor model used for the receptor-mediated ligand alignment were discarded.

In the next step, we constructed a peptidic minireceptor consisting of nine key amino acid residues (Arg, His, Ala, Tyr, Ser, Trp, Thr, Phe and Ile), interacting with the various functional groups of the 17 sweet compounds of the training set. The residual binding site was then completed by virtual particles (r = 0.80 Å, well-depth ε = −0.024 kcal mol^{-1}). The virtual particles represent 'unspecific hydrophobic regions' of the receptor surrogate and are automatically generated by the program using a van der Waals surface algorithm. They are only placed in regions where no specific amino acid residues occur. In this model they numbered 139. In contrast to the amino acid residues they are not electrically charged.

This complex was then optimised using *ligand equilibration*, a

§ The algorithm of Marengo and Todeschini was originally developed for applications to distance-based experimental design with the aim to select a fraction of most different compounds from a given set of molecules by means of the maximal 'minimum distance'. In the approach of Zbinden and co-workers the 'minimum distance' between two molecules is computed as a weighted function of electrostatic, van der Waals and H-bond interactions, determined at a 'primordial' receptor model or, alternatively, at points of a common surface (*e.g.* a van der Waals envelope).

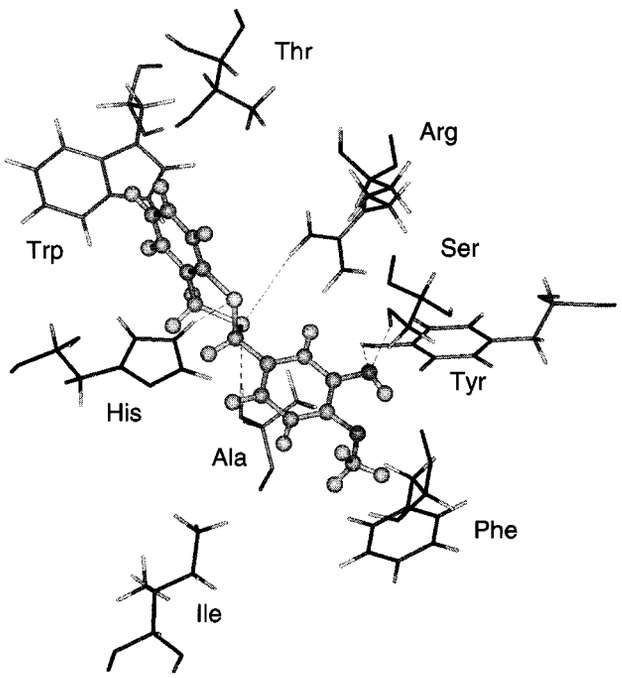


Fig. 6 The three-dimensional structure of the pseudoreceptor model for sweet isovanillic derivatives. Only some interactions are shown.

protocol where *correlation-coupled receptor optimisation* and free ligand relaxation are altered in an iterative fashion until a high correlation is obtained in the relaxed state.[22] During free ligand relaxation, we included a Monte-Carlo search [250–750 generated conformers per sweet compound, differing at least by a root mean square (rms) deviation of 0.5 Å; 25 thereof were fully relaxed during each of the eight equilibration rounds].¶ This procedure yielded a correlation coefficient [r for eqn. (3)] of 0.956 and an rms deviation of experimental and predicted free energies of ligand binding, $\Delta G°$, of 0.208 kcal mol^{-1}, corresponding to an uncertainty factor of 1.4 in the relative sweetness (Table 1).

The model (Fig. 6) can be best described as a hydrophobic cavity (Phe, Trp, Ala, Tyr, His; hydrophobic particles) with distinct H-bonding sites (Arg, His, Tyr, Ser, Thr and Ala *via* its main-chain N atom). For the sweetest compound of the series, **3**, this leads to the following interaction scheme: one S atom of the six-membered ring engages in two hydrogen bonds with the Arg residue (3.07 Å) and the main-chain N atom of the Ala residue (2.67 Å). The other interacts similarly with the imidazole moiety of the His residue (2.84 Å) and the other guanidinium N atom of the Arg residue (3.37 Å). The phenolic hydroxy O atom engages in two hydrogen bonds with the Ser (2.13 Å) and the Tyr residue (2.12 Å), respectively. The phenolic hydroxy H atom forms an intramolecular hydrogen bond with the O atom of the vicinal methoxy group. The Thr residue has no H-bond contacts with **3**, but interacts with **13** and **21** instead.

To analyse the predictive power of the model, the eight sweet compounds defining the test set (not part of the training set) were added to the receptor model and subjected to a free ligand relaxation including a Monte-Carlo conformational search (settings as performed for the training set). This yielded an rms deviation of experimental and predicted free energies of ligand binding, $\Delta G°$, of 0.994 kcal mol^{-1}, corresponding to an uncertainty factor of 5.5 in the relative sweetness (Table 1). The largest individual deviation was observed for **16** which was predicted as being 18 times too sweet, while all others are predicted within the same order of magnitude as the experiment. Therefore, a factor of approximately 10 in the binding affinity (or relative sweetness) might currently be considered as a realistic accuracy of the method.

¶ During the Monte-Carlo search position, orientation and conformation are altered. The RMS deviation implies that a new position, orientation and conformation is accepted (for minimization) only if it deviates at least 0.5 Å from its parent structure. This way prevents refining a large number of almost identical structures during the conformational search.

Conclusions

The first three-dimensional receptor model for a class of sweet compounds was constructed. Although the lack of knowledge about the actual peptidic receptor(s) prevents any comparison with the model, this latter may be of heuristic value in the design of new sweet compounds of the isovanillic group. Investigations on the extension of the model to encompass other classes of sweet compounds is in progress.

Acknowledgements

The authors wish to thank Dr Enzio Ragg (DISMA, University of Milano) and Dr Michael G. B. Drew (University of Reading, UK) for helpful discussions. Financial support by the EC (contract AIR3-CT94-2107) and the Foundation Biographics Laboratory 3R, Basel, Switzerland is gratefully acknowledged.

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Paper 7/09167B
Received 22*nd December* 1997
Accepted 7*th April* 1998