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Isovanillyl sweeteners. From molecules to receptors*

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Abstract: Systematic modification of the structure of the sweet natural compound phyllodulcin, containing the isovanillyl glucophoric group, led to the synthesis of about 120 compounds. Features of the heterocyclic ring conferring high sweetness potency were identified. A strong increase in sweetness was obtained by the introduction of sulfur atoms in the ring and by separation of the enantiomers. Results of the quantitative structure–activity relationship (QSAR) studies on this series are reported. Application of the pseudoreceptor modeling approach afforded a three-dimensional binding site model for isovanillyl sweeteners. Extension of this methodology to a large group of structurally diverse compounds, including the commonly used sweeteners, gave a general pseudoreceptor for the sweet compounds, consisting of 16 amino acids. This pseudoreceptor, which has the peculiarity of giving a semiquantitative evaluation of the sweetness intensity, could be used as a valid tool to model the ligand–receptor interactions and to suggest some clues about the identification of a possible binding site once the receptor protein(s) are obtained.

In the continuous search of chemists for new potent biologically active compounds for many applications, Nature has been the main source of inspiration. This also holds true, at least in part, for sweeteners. In fact, if a series of the presently used sweeteners have been discovered serendipitously, others have been found by starting from natural substances and making patient and systematic modifications.

The so-called isovanillyl sweet compounds owe their name to the presence of the isovanillyl (3-hydroxy-4-methoxyphenyl) group in some sweet natural compounds, such as phyllodulcin (**1**) and neohesperidin dihydrochalcone (**2**, NHDC). Phyllodulcin, isolated by the renowned Japanese natural product chemist Asahina in 1916 from *Hydrangea opuloides* Steud. Var. Thunbergii, MAKINO [1], is responsible for the sweet taste of an infusion used in Japan, whereas NHDC, a well-known commercial sweetener, results from slight modification of neohesperidin, a constituent of orange peel. Other sweet isovanillyl natural compounds (e.g., **3** and derivatives) were isolated by Kinghorn in 1988 from *Tessaria dodoneifolia* [2] (Fig. 1).

The remarkable sweetness potency (relative sweetness with respect to sucrose, RS being 600–800 for **1**, 350–400 for **2** and 400 for **3**) of these compounds was attributed to the presence of the common isovanillyl glucophore, whose characteristics of having two adjacent hydrogen-donor and hydrogen-acceptor groups satisfy the requirements of the Shallenberger–Acree–Kier [3] model (Fig. 2). A first, but isolated, attempt to develop a synthetic sweet compound based on the isovanillyl group was made by Dick in 1981 [4], who prepared compound **4** (RS 3000, Fig. 3), but its instability in aqueous media most probably prevented the authors from pursuing this investigation.

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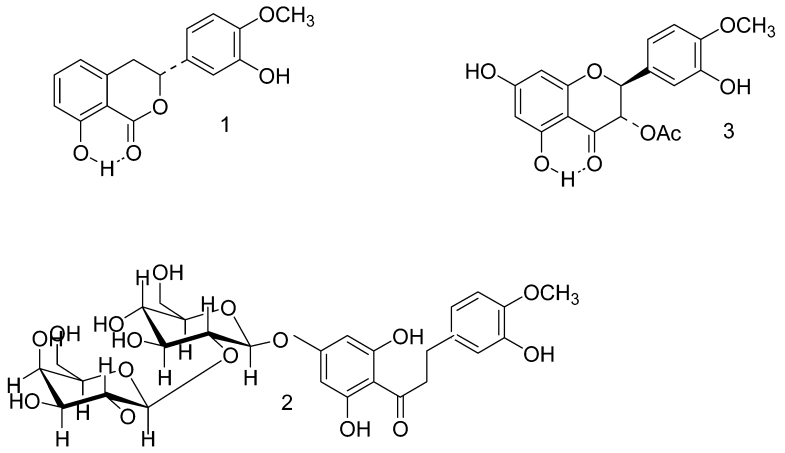


Fig. 1 Structure of isovanillyl sweeteners.

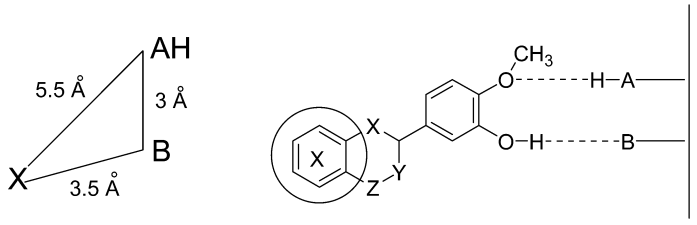


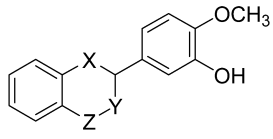
Fig. 2 The Shallenberger–Acree–Kier model and its application to isovanillyl compounds.

Fig. 3 Structure of 1,3- and 1,4-benzodioxane isovanillyl sweet compounds.

Our interest in this field began with the synthesis of the corresponding, much more stable, and remarkably sweet 1,4-benzodioxane analog (**5**, RS 450, Fig. 3) [5].

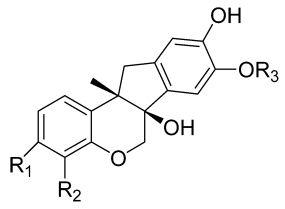
In order to develop structure–activity relationships (SARs) to better understand the requirements of the receptor to which the sweet compounds were supposed to bind, a systematic study of the structural and stereochemical features of this class was undertaken (Fig. 4).

The synthesis of compounds with a 5-, 6-, and 7-membered heterocyclic B ring, and with one or two oxygen atoms in all the possible positions (Fig. 4) led to the conclusion that greater sweetness was correlated with a 6-membered ring and with two oxygen atoms in position 1,3. NMR studies and molecular mechanics calculations indicated a preferred conformation with the isovanillyl C ring almost perpendicular to the plane of the AB rings, with a twist angle of ca. 25° [6]. Attempts to capitalize on this indication led to the synthesis of a series of rigid compounds, none of them, however, being particularly sweet [7]. An interesting case of rigid compound is again that of a natural compound, (+)-haematoxylin (**6**), a well-known constituent of *Haematoxylon campechianum*, that is sweet, as well as one of its isovanillyl analogs (**7**, Fig. 5) [8].



X	Y	Z	RS
O	CH_2	CH_2	350
CH_2	CH_2	O	200
CH_2	O	CH_2	350
O	O	---	150
O	O	CH_2	3000
O	CH_2	O	450
S	CH_2	CH_2	200
S	O	CH_2	9000
S	S	CH_2	10000
O	S	CH_2	500
O	CH_2	S	250
S	CH_2	S	500
O	O	$(CH_2)_2$	0

Fig. 4 Structure and sweetness (relative to 3 % aqueous sucrose) of isovanillyl compounds.



6 R_1 = OH R_2 = OH R_3 = OH

7 R_1 = H R_2= H R_3 = OCH_3

Fig. 5 Structure of (+)-haematoxylin and of a sweet derivative.

Whereas no derivative appeared sweeter than **4**, a strong increase in the sweetness potency was obtained when one or two sulfur atoms were substituted for the oxygens of the heterocyclic ring, leading [9] to the sweetest compounds of the series, the 1,3-benzoxathiane **8** and the 1,3-benzodithiane **9** (RS 9000 and 10 000, respectively) (Fig. 6). Although positive effects of sulfur groups on sweetness had been observed previously [10], a rationalization of this effect in the case of the isovanillyl derivatives is not easy. Most probably it derives from the increase of the dipolar interaction with a receptor site, due to the greater size and polarization of the sulfur atom with respect to oxygen.

If the sweet taste is the result of an interaction of the tastant with a proteic receptor, there is no doubt that this occurs in a chiral environment, and, therefore, chirality must play an important role among the requisites for the interaction. As most, and among them the sweetest, isovanillyl derivatives possess a stereogenic center, a difference in the sweetness potency of the two enantiomers was expected. Resolution of the racemic **8** and **9** by chiral high-performance liquid chromatography (HPLC) gave a sweet (RS 18 000 and 20 000, respectively) and a tasteless enantiomer, the sweet enantiomers possessing the configuration *R* (Fig. 6) [11]. This was consistent with the lack of sweetness of the "unnatural" *S* enantiomer of phyllodulcin, synthesized by Gerlach [12].

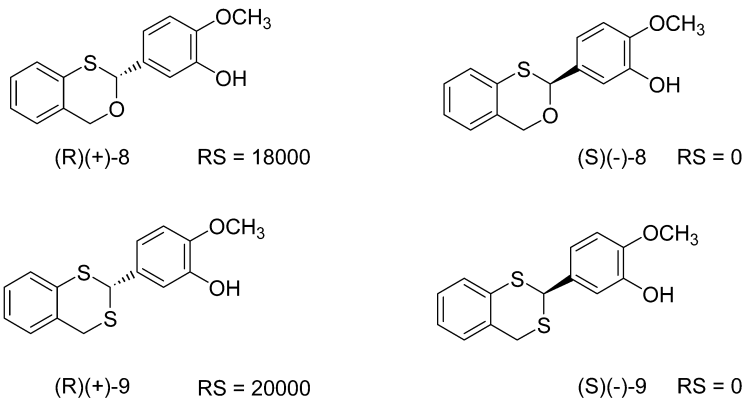


Fig. 6 Relationship between sweet taste and configuration in two isovanillyl compounds.

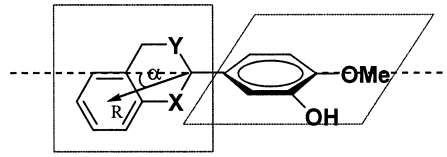


Fig. 7 Geometrical parameters used for SAR studies of isovanillyl compounds.

The wealth of data collected in this series prompted us to explore structure–taste relationships. A first empirical analysis in the oxygen series allowed us to identify some geometric parameters (Fig. 7). Most of the sweet compounds had values for these parameters within a definite range [6].

With the increased number of data available, a statistical approach to QSAR was undertaken, using the genetic algorithm method and molecular field analysis. This resulted in a series of equations, the most reliable of them showing dependence of sweetness on a few parameters correlated with lipophilicity and electronic properties [12,13].

Another important outcome of such research could be obtaining information about the interaction of a sweet compound with the putative receptor for the sweet taste. Until very recently (2001), not only was nothing known about the structure of such a receptor (or receptors), due to the failure of attempts to isolate it from human or animal tissues, but there was also no perspective of obtaining such information in a short time. The only progress toward this goal has been the structural elucidation of antibodies developed against guanidinic sweeteners [14]. There was, thus, scope for pursuing the development of glucophore models stemming from the structure–taste relationship.

The simple but very helpful Shallenberger–Acree–Kier model (Fig. 1) dominated the scenario for 20 years, until the discovery of the hyperpotent guanidinic sweeteners allowed Nofre and Tinti [15] to propose a more sophisticated and detailed model (Fig. 8).

In the meantime, progress in nuclear magnetic resonance, theoretical structural chemistry, and calculation of molecular properties stimulated the application of these methods in the field, resulting in the Temussi–Goodman [16] model derived from aspartame, and in the Walters similar approach starting from guanidinic sweeteners [17].

However, none of these models could give an at least semiquantitative correlation of sweetness with structure, nor gave hints about the possible structure of the receptor. To address these issues, a pseudoreceptor modeling approach was used. In this approach [18], the properties of the bioactive lig-

ands are projected into three dimensions around their appropriately superimposed molecular framework. The resulting map provides steric, electrostatic, and lipophilic profiles (anchor points), which can be used to identify the type and approximate position of residues, or their functional groups, in the true biological receptor that interacts with the ligands. This map can be used for subsequent molecular modeling and allows semiquantitative predictions of binding affinities for ligands. Application of this method to isovanillyl sweet compounds using a group of 17 compounds to build the pseudoreceptor and a group of 9 ligands as a test set afforded a three-dimensional binding site model of the sweet receptor constituted by 9 amino acids [19]. This model correctly predicted the interaction energy, and, consequently, the sweetness potency, of the test set of compounds (Fig. 9).

With the hope of extending this methodology to a larger group of compounds, a similar pseudoreceptor was built for the guanidinic sweet compounds, using a training set of 39 compounds and a test set of 8 compounds. Results were even better, most probably due to the structural homogeneity of the compounds investigated. These encouraging results prompted the search for a general pseudoreceptor that could accommodate the great structural variety of the currently used sweeteners and of many sweet compounds belonging to diverse chemical classes. The main difficulty in this case was the critical cri-

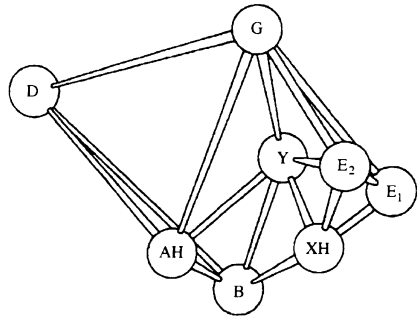


Fig. 8 The Nofre–Tinti model.

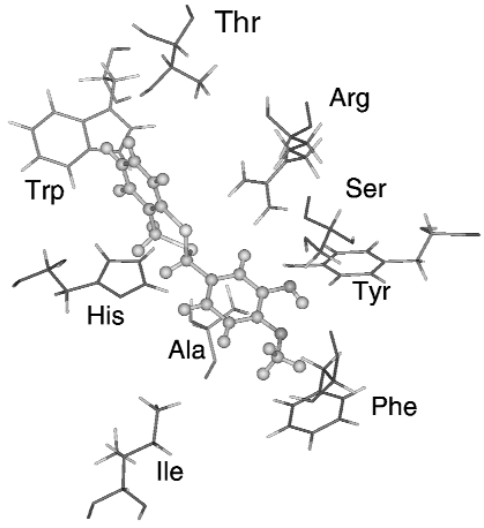


Fig. 9 The pseudoreceptor for isovanillyl compounds.

terium of superimposition of different ligands. This difficulty was overcome by taking advantage of the preceding empirical glucophoric models, and thus the ligands (including sucrose, sucralose, aspartame, saccharin, isovanillyl compounds, guanidinic compounds, monatin, etc.) were superimposed using as a template the Nofre–Tinti model. As a result, a general pseudoreceptor for the sweet-tasting compounds was obtained, consisting of 16 amino acids (Fig. 10). The correlation between the predicted and the measured sweetness potency for a test set of 5 compounds (neotame, an isovanillyl and a guanidinic derivative, 6-chlorotryptophan and a sugar) was excellent (Fig. 11). The model was further validated using a randomization test.

The model thus obtained is able to explain and predict the sweet taste of compounds belonging to different families. Its structural features are in agreement with the preexisting models suggested for the sweet taste receptor, but its peculiarity is to give a semiquantitative evaluation of the activity. The recent discovery of a putative sweet taste receptor gene [20] strengthens the hypothesis of the existence of a G protein-coupled receptor (GPCR)-mediated chemoreception mechanism for sweet tastants. However, only preliminary hypotheses are available about the structure and function of this protein(s), and, there-

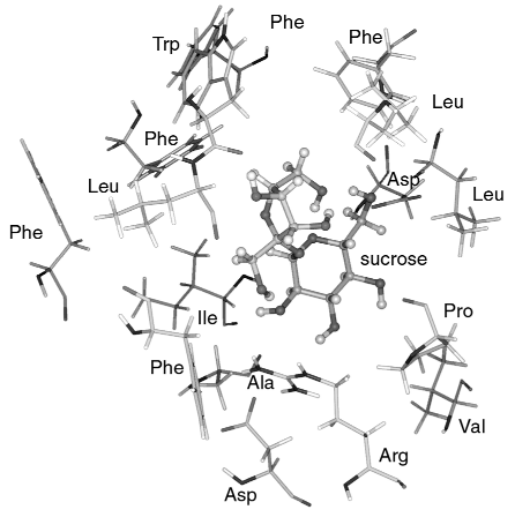


Fig. 10 The general pseudoreceptor for sweet compounds.

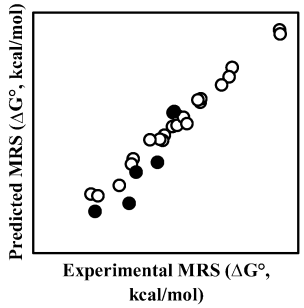


Fig. 11 Correlation between experimental and predicted sweetness according to the pseudoreceptor model of Fig. 10.

fore, alternative methodologies are still needed to explain the taste of known compounds and to predict the taste of new derivatives. The pseudoreceptor model seems to fulfill both these requirements, and it could, therefore, be used as a valid tool to model the ligand–receptor interactions and to suggest some clues about the identification of a possible binding site once the receptor protein(s) are obtained.

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