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Progress in isovanillyl sweet compounds

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The so-called isovanillyl sweeteners are organic compounds containing the 3-hydroxy-4-methoxyphenyl moiety, which seems to be essential for the sweet taste of these compounds. Most of them are heterocyclic compounds, with a free rotating isovanillyl group and one chiral carbon atom. Rigid compounds with structures mimicking some of the possible preferred conformations which can be assumed by the isovanillyl sweeteners were prepared and tasted. To establish possible correlations between chirality and taste, the enantioselective synthesis of the (−)-enantiomer of the natural sweet compound (+)-hematoxylin was performed. The sweetest among the isovanillyl compounds was modified by introducing groups apt to interact with the D site of the Nofre–Tinti receptor model. All the results are discussed on the basis of comparison with the most recent models for the sweet taste receptor. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The term 'isovanillyl sweeteners' designates a class of sweet compounds which contain the 3-hydroxy-4-methoxyphenyl moiety. Members of this group are the natural substances **1** (phyllodulcin) and **2**, the semi-synthetic commercial sweetener neohesperidin dihydrochalcone (NHDC) and **3**, a large number of synthetic products, most of them prepared using **1/2** as leading models (Scheme 1).

The two essential features which seem to confer sweetness on these compounds are the *ortho*-hydroxymethoxy unit, and another aromatic ring, which constitute the AH–B and the hydrophobic G group, respectively, according to the classical Shallenberger–Acree–Kier model of the sweet taste receptor (Shallenberger, 1992).

Preceding work in this area, including our own (Arnoldi *et al.*, 1992*a*,*b*) has mostly involved exploration of the structure, size, geometry, and other physicochemical characteristics of the link between these two moieties, which in most cases is a heterocyclic ring. Tasting of the compounds prepared has shown that increasing sweetness potency appears in compounds with a six-membered heterocyclic ring, with two oxygen or sulfur atoms in positions 1 and 3 (general structure **4**), the maximum (9000 times with respect to sucrose) being reached in compound **5**, with sulfur in position 1 and oxygen in position 3 (Scheme 2) (Arnoldi *et al.*, 1993).

Therefore these atoms are not only a link between the two glucophoric moieties, but must also interact with some corresponding polar site of the receptor. This evidence has not yet given rise to an adaptation of the existing models of the sweet receptor structure to this class of sweet compounds.

In a preceding paper (Arnoldi *et al.*, 1992*a*) we have emphasized (Fig. 1) the structural and conformational properties of the isovanillyl sweet compounds, which allow a certain range of incertitude in the spatial definition of the interaction sites with the receptor.

A first approach to these problems was the synthesis of simple rigid compounds with structures which could mimic some of the possible conformations accessible to the heterocyclic ring by rotation around the bond linking this moiety to the isovanyllic ring. Scheme 3 shows some of these compounds (**6–11**). Only **8** and **11** appear slightly sweet. This result would indicate the preferred interaction of a free-rotating ring in **4** oriented as in **11** rather than in **9** and **10**. This is consistent with the Shallenberger–Acree–Kier model. If the triangle AH–B–G is positioned in order to have AH–B on an axis, say y (Fig. 2), the hydrophobic region must lie in the quadrant $+x$, $+y$, as it indeed occurs in **11**. This holds also for **6**, whereas the difference between **7** and **8** appears to be more subtle. Here the reason for the difference in sweetness may be the less favourable position of the cyclopentane ring in **7**, or the effect of the bulky angular methyl group, which protrudes perpendicularly to the main plane of the molecule (and of the receptor) in opposite directions in **7** and **8**, and could interfere with the so-called Shallenberger barrier.

The small value of the sweetness potency of all these compounds can be ascribed to their scarce hydrophilicity, and to the absence of polar atoms able to

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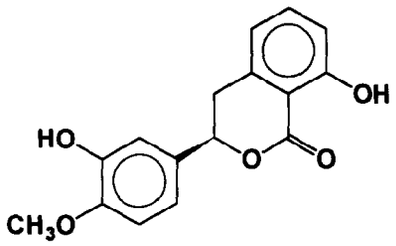
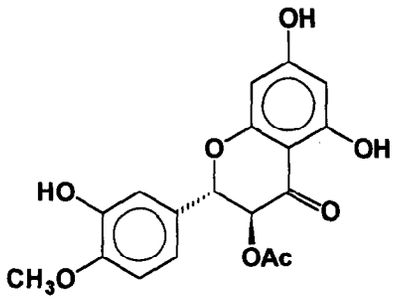
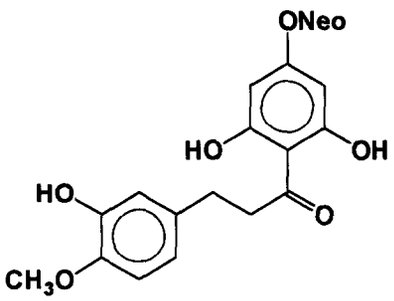


(+)-1 400x



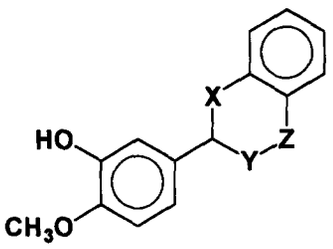
2 80x



Neo = -L-Rhamno-ß-D-glucopyranosyl-

3 600x

Scheme 1



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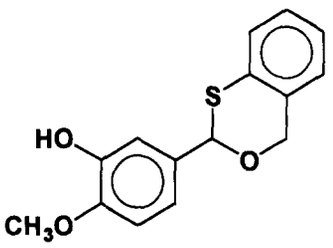


5

X, Y, Z = $(CH_2)_n$, O, S

9000x

Scheme 2

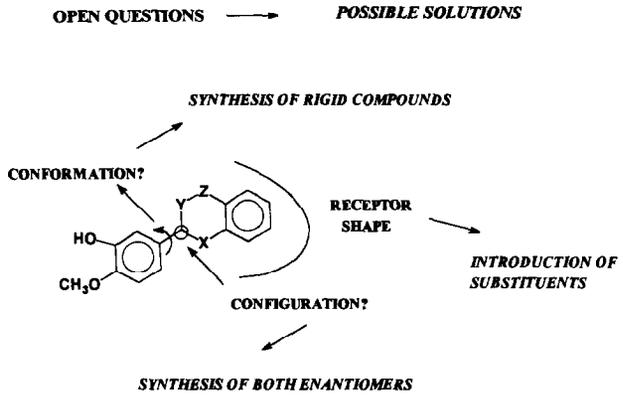
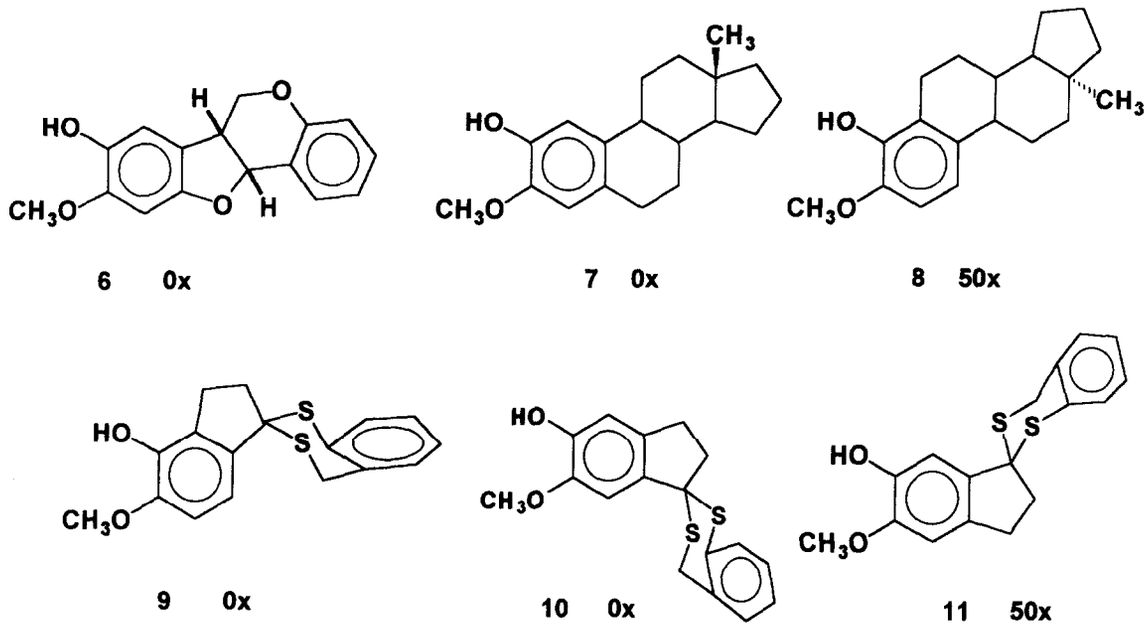


Fig. 1. Current topics of study among the isovanillyl sweeteners.

confer additional possibility of interaction. Further work directed to the synthesis of structurally similar, more hydrophilic compounds and with polar atoms in the appropriate positions, is in progress, although hampered by synthetic difficulties.

Another topic of interest in this area is the relation between chirality and sweet taste. Phyllodulcin **1** is a chiral compound, of which only the natural (+)-enantiomer is known. Attempts to synthesize the (−)-enantiomer have so far failed (Arnoldi *et al.*, 1992*b*), whereas all the sweet compounds of general structure **4** have been obtained as racemates. Work is in progress toward the enantioselective syntheses of other compounds of this class.



Scheme 3

Another long-known natural substance of a similar structure, which however was only recently reported to be sweet (Masuda *et al.*, 1991) is (+)-haematoxylin **12** (Scheme 4).

This report raised our interest, as haematoxylin is a fairly rigid compound, exhibits different groups capable of an AH–B type interaction with the receptor, and is a chiral compound. Rigidity facilitates comparison with models of the sweet receptor, without uncertainties due to conformational mobility. In haematoxylin both *ortho*-dihydroxy groups can act as AH–B groups, and in two modes. In the attempt to identify the AH–B group best suited to confer sweetness to haematoxylin, we have prepared a series of methylated isomers [**13–16**, Scheme 5 (Arnoldi *et al.*, 1995)] where the *ortho*-OH-OMe moiety can act in only one definite mode as an AH–B group. Only one (**13**) of these derivatives is sweet, which would indicate that, if this group acts as an AH–B group, the remaining part of the molecule is oriented so as to give a positive interaction with the receptor.

Therefore the same orientation should be assumed by haematoxylin. A systematic comparison of these structures with the most recent and detailed model of the receptor, proposed by Nofre and Tinti (Nofre & Tinti, 1992) seems to support the preceding assumption. Only the orientation shown in Fig. 3, corresponding to that allowed to compound **13**, brings the aromatic ring A to occupy the zone of interaction with the hydrophobic site G. (This analysis also takes into account, and excludes, the ethereal oxygen atom in position 5 and the OH in position 6a from acting as B and AH groups, respectively.)

The second interesting feature of haematoxylin, its chirality, has been exploited through the enantioselective synthesis of the enantiomer (−)-haematoxylin (−)-**1** (Arnoldi *et al.*, 1995). This compound appeared about 50 times sweeter than sucrose, against the ≈120 times scored by the natural enantiomer. Figure 4 shows the superimposition of the two enantiomers, seen in a plane corresponding to the main dimension of the receptor (Fig. 4a), whereas the differences are larger in the perpendicular direction (Fig. 4b), where the steric requisites of the receptor are less stringent or defined. However, the difference in the sweetness potency is too low to be of any significance in this respect.

The recent outstanding results obtained by Nofre and Tinti in the design of new hyperpotent sweeteners have

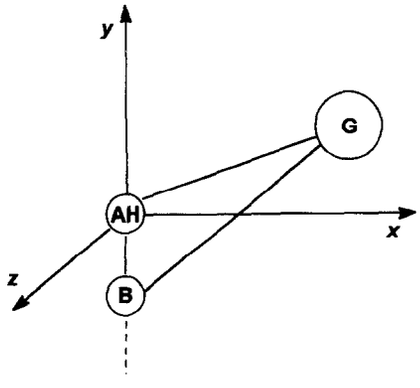
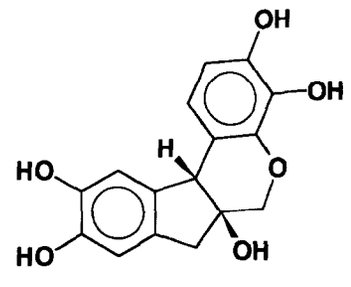
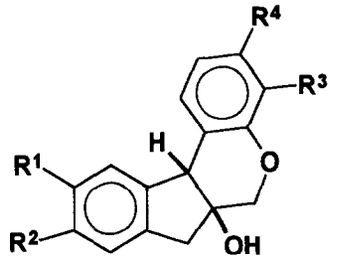


Fig. 2. The Shallenberger–Kier–Acree model for a sweet compound.



(+) **12** 120x

Scheme 4



Comp.	R1	R2	R3	R4	Taste
13	OH	OMe	H	H	50x
14	OMe	OH	H	H	0x
15	OMe	OMe	OH	OMe	0x
16	OMe	OMe	OMe	OH	0x

Scheme 5

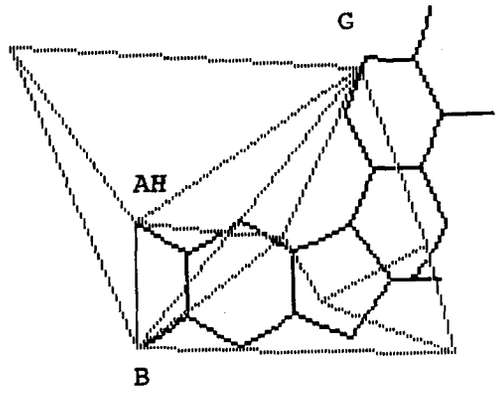


Fig. 3. Superimposition of compound **13** and of a schematized Nofre–Tinti model.

culminated in the synthesis of the guanidinic sweet compounds. A consequence of this success was the formulation of a new model of the sweet taste receptor, with at least eight possible sites of interaction (Nofre & Tinti, 1992).

The availability of the Nofre–Tinti receptor model, which includes the D site of interaction, corresponding to the *para*-cyano- or *para*-nitrophenyl group, not taken into account by preceding proposals, prompted us to compare the general structure **4** with this model, with the aim of improving its adherence to the model. If isovanillyl compounds owe their sweetness mainly to the presence of the AH–B and G sites, the improvement of the sweetness potency or quality by introducing a new structural unit corresponding to the D group appeared as an attractive goal.

We report here on the first attempts in this direction, the routes chosen for a first rough exploration of the field not being exceedingly exacting in terms of synthetic procedures. An obvious starting material appeared to be compound **5**, the sweetest of the isovanillyl series, on which could be appended a suitable flexible chain carrying the glucophoric *p*-nitrophenyl group. Examination of the structure **5** in comparison with the model indicated (as a possible target) a compound with a 3-atom chain linked to carbon 2′ of the isovanillyl ring, in order to bring the new D-type group to the distance of about 10 Å from G, demanded by the model. However, the synthesis of the vicinally tetrasubstituted benzaldehydes, as necessary intermediates, appeared to be quite demanding; so more easily attainable subtargets appeared to be compounds with the new D-type group linked to the OH of the isovanillyl group. This operation would eliminate the AH group, with a certain negative consequence on the total sweetness, as expected by the comparison of **5** (9000×) with **17** (tasteless), but on the contrary could furnish useful information on the effect of the newly attached D-type group with a limited synthetic effort. Scheme 6 shows the compounds prepared (**17–24**) and their sweetness potency with respect to 3% sucrose. The increase from **19** to **21** and the sweet taste of compound **23** (lacking the B group) are consistent with the role played by a D-type group, whereas comparison of **22** with **21** again is a proof of the importance of the secondary interactions of the 1,3-heteroatoms, and particularly of sulfur in position 1. The difference between **24** and **21** might depend on small differences in the conformation of the chain. A

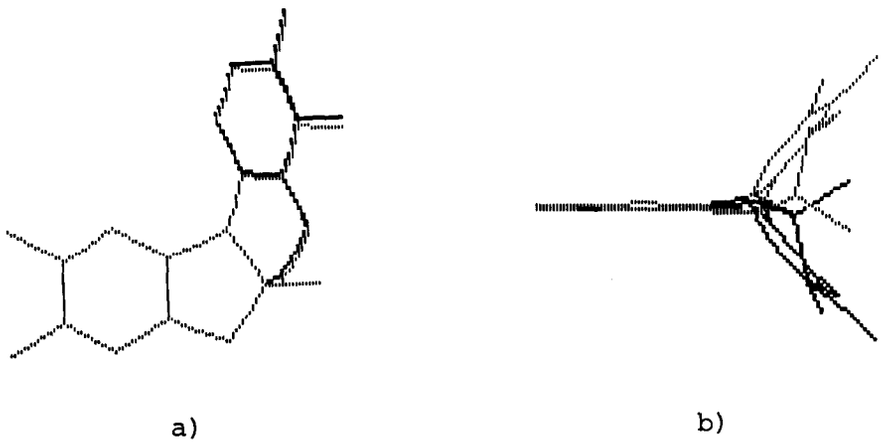
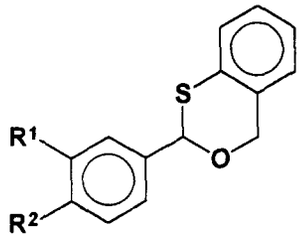


Fig. 4. Two orthogonal views of the superimposition of (+)- and (−)-haematoxylin.

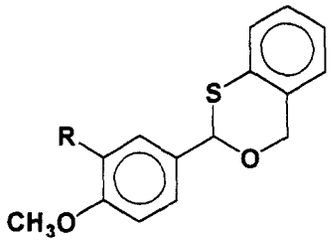
possible, still tentative, explanation of the general decrease in sweetness potency from **5** would be that in the class of the isovanillyl compounds the *ortho*-CH_3O-C=C-OH moiety is by and large the most important for the interaction with the receptor, as indirectly shown by the dramatic decrease of potency from **5** to **17** and to **18**.

We reverted then to the original aim of introducing the D-type chain on the 2′ carbon of the isovanillyl ring (an operation that would maintain the *ortho*-OH-OMe moiety), taking advantage of the fact that 2-amino-3-hydroxy-4-methoxybenzaldehyde could be prepared in a satisfactory yield. Having been confronted with failures in the derivatization of the amino group into *p*-nitrophenyl(iso)thiocyanate, we have been able so far to obtain the amide **25** (Scheme 7), that is, however, tasteless.

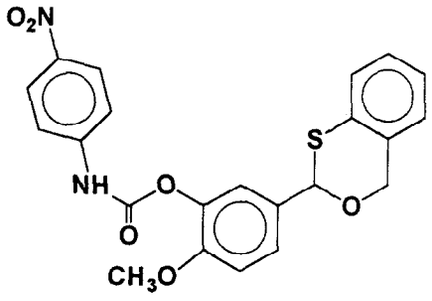
Two other compounds with a similar side-chain, **26** and **27**, were prepared by Heck reactions and only **26** is



	R^1	R^2	Taste
5	OH	OMe	9000x
17	H	OMe	0x
18	OH	H	120x

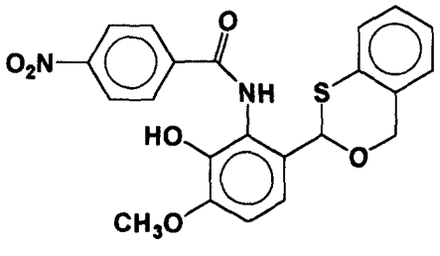


	R	Taste
19	EtNHCOO	0x
20	PhNHCOO	600x

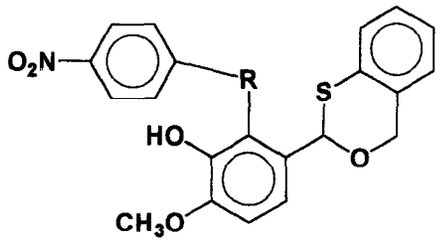


21 1100x

Scheme 6



25 0x



	R	Taste
26	CH=CH	sl. sweet
27	CH_2-CH=CH	0x

Scheme 7

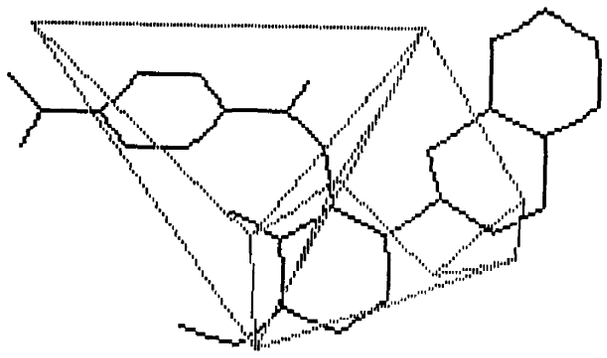


Fig. 5. Superimposition of compound **25** and of a schematized Nofre–Tinti model.

slightly sweet. At present, only speculations are possible to explain the absence of sweetness in these compounds, which still maintain the AH–B group. However, they are offered here just to show how complex is the detailed interpretation of structure–taste relationships, even for a rather homogeneous series of compounds. Molecular mechanics studies indicated the possibility of unexpected conformations of low energy, with the *p*-nitrophenyl ring almost parallel to the aromatic ring, and therefore far away from the D site. Stacking interactions could favour this conformation. On the other hand, favourable interactions with the D site could be reached by other (also low energy) conformations. If we compare **25** with the Nofre–Tinti model (Fig. 5), we find

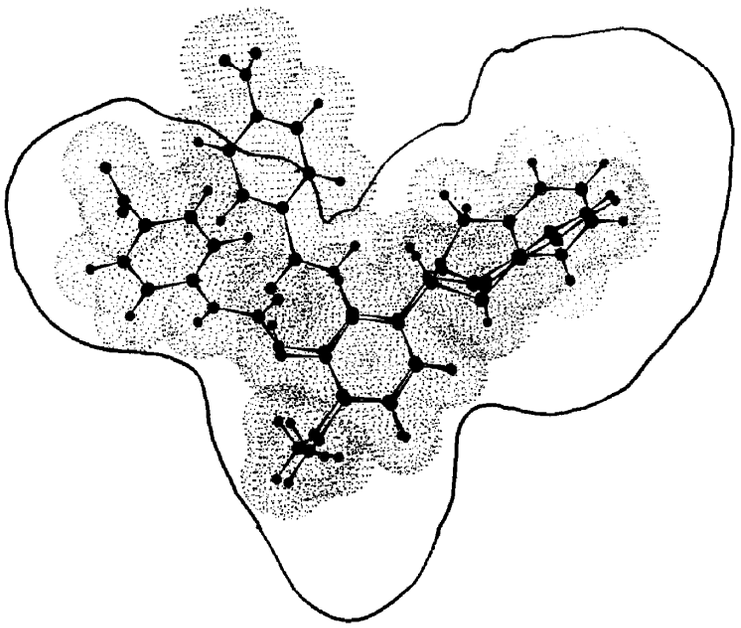
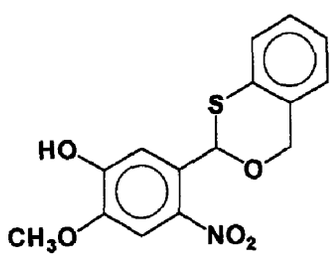


Fig. 6. Superimposition of compounds **21** and **25** and of a projection of the DuBois–Walter–Kellogg model.



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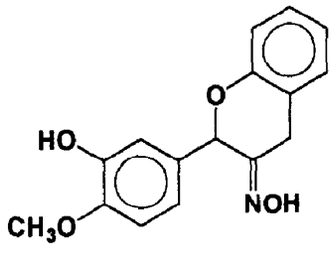

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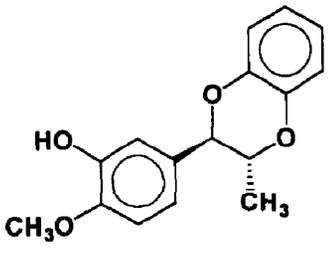

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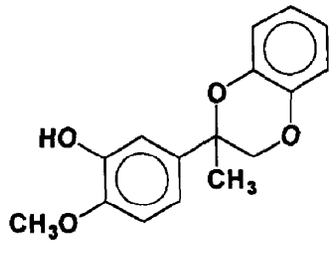

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Scheme 8

possible positive interactions with sites AH, B, G and D. However, the linking arm with the D-group falls in a region of the receptor not occupied by the Y-shaped model of Nofre–Tinti and therefore unknown as concerns its steric requirements. It is also noticeable that, although in **25** the distance between the *p*-nitrophenyl group (D-type) and the aromatic ring (G-type) is around the 10 Å indicated by the Nofre–Tinti model, the overall shape of **21** fits much better, at least qualitatively, than **25**, with the cavity of the DuBois–Walters–Kellogg (DuBois *et al.*, 1992) model also based on the guanidinic sweeteners (Fig. 6).

Another independent explanation of this failure might be the presence of a bulky substituent adjacent to the isovanillyl ring, which can occupy a forbidden region of the receptor, or strongly hinder the rotation of the ring, thereby preventing the compound from reaching the active conformation. As a matter of fact, there are other examples of compounds with groups in such a position which are tasteless (e.g. **28–31**; Scheme 8).

None of these possible explanations is well substantiated which means that further accurate work to collect structure–taste relationship data is necessary to further refine the shape of the receptor for this class of compounds.

ACKNOWLEDGEMENTS

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