Sweet Isovanillyl Derivatives: Synthesis and Structure−Taste Relationships of Conformationally Restricted Analogues

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In structure−taste relationships of sweet substances, conformational analysis is important for the definition of the "active conformation" which actually interacts with the sweet taste receptor. This paper describes the synthesis and taste of some isovanillyl derivatives having a rigid structure, which can mimick only one possible conformation of their flexible analogues. We report also the solid state conformation of a sulfur-containing sweet isovanillyl derivative which was established by X-ray analysis. The relationships between conformation and taste were studied by comparison with the current models of the sweet taste receptor and by principal components analysis (PCA) using geometrical descriptors, followed by factor and cluster analysis. A strong correlation was proven between taste and geometry, which is able to explain the taste of rigid derivatives as well as the difference of taste between the two enantiomers of the same compound.

Keywords: *Sweetness; taste; isovanillyl derivatives; conformational analysis; SAR (structure−activity relationships); PCA (principal components analysis); factor analysis; cluster analysis*

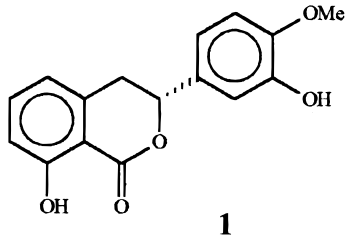
INTRODUCTION

The general features of the sweet taste receptor have been extensively studied by several authors. The first general model was proposed by Shallenberger and Acree (1967) and Kier (1972), and it consists of three interaction points called AH, B, and X (or G). This simple model has been successfully used by several authors (Lee, 1987; Van der Wel et al., 1987) to explain, at least in part, the sweet taste of several sweet compounds. More recently an important contribution to the mechanistic understanding of sweet taste has been given by Nofre and co-workers (Tinti and Nofre, 1991; Nofre et al., 1996) with their multipoint attachment theory.

Two major contributions to the study of the geometrical features of the receptor are due to the work of Temussi (Temussi et al., 1978, 1991; Ciajolo et al., 1983) and Goodman groups (Douglas and Goodman, 1991; Yamazaki et al., 1991, 1994), who independently derived a tridimensional map of the active sites cavity in the sweet taste receptor by conformational studies of flexible peptides and peptidomimetic compounds, obtained either by X-ray analysis, NMR experiments, or calculations (Figure 1). Also some rigid derivatives have been used as "molecular molds" (Castiglione Morelli et al., 1990). The two models substantially agree in describing the active site as a flat cavity, lying mainly in the *x*, *y* plane (*y*, *z* for Temussi). Only molecules with a small extension along the +*z* axis (−*x* for Temussi) are able to elicit a sweet taste, owing to the presence of the so-called Shallenberger barrier, at a distance of about 0.3 nm from the *x*, *y* plane. Molecules that project long hydrophobic chains toward the −*z* axis are bitter.

Both authors agree on the need of an almost complete coplanarity of the AH−B system and the hydrophobic G moiety which often corresponds to an aromatic or aliphatic ring. For Temussi, the planarity of the G region in the *x*, *y* plane is so important that sometimes a distortion of the AH−B groups is accepted (Castiglione Morelli et al., 1990). For the construction of their model, Goodman and his group started from the solid state conformation of aspartame and imposed a rotation of 40 degrees about the $\Phi_{(Phe)}$ in order to obtain an isoenergetic conformation in which the phenyl ring and the AH−B moiety are coplanar (Douglas and Goodman, 1991). On the other hand, this conformation is preferred in solution (Castiglione Morelli et al., 1990).

Isovanillyl sweeteners are a large class of derivatives structurally related to the natural compound (+)-phyllodulcin **1** (Asahina and Asano, 1929) and to the semisynthetic sweetener neohesperidin dihydrochalcone (NHDC) **2** (Horovitz and Gentili, 1969).



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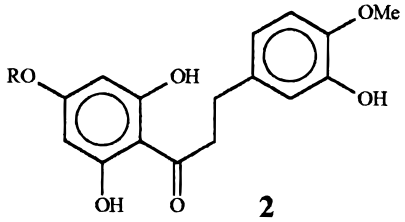

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R = 2-O-α-L-rhamnosyl-β-D-glucopyranoside

Preceding work in this area including our own (Arnoldi et al., 1991, 1993, 1996) has focused on the

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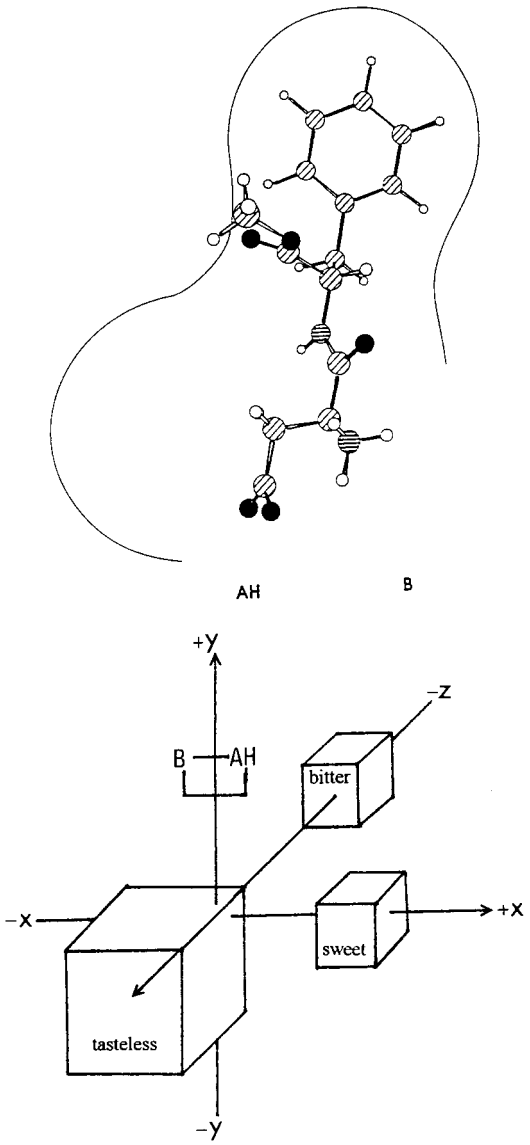


Figure 1. Receptor models of Temussi and Goodman groups.

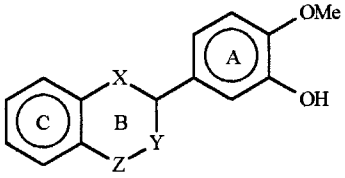


Figure 2. General structure of an isovanillyl derivative.

importance of the size of the heterocyclic ring, the type and number of heteroatoms, and the identification of the possible glucophores by comparing the molecular structure with the existing models. The 3-hydroxy and 4-methoxy substituents and the aromatic ring A are generally believed to correspond respectively to the binding sites AH, B, and G (Figure 2).

A particular issue is the relationships between taste and conformation of these compounds. In fact, flexible sweet substances can adopt many possible conformations during the interaction with the receptor, only one of them being presumably the active one.

The conformation of (+)-phyllodulcin **1** in the solid state is not known. DuBois et al. (1977) suggested a bent conformation as the active form for this derivative and for NHDC, on the basis of the fitting of this conformation with the distances between the three interaction points AH, B, and X in the Shallenberger–Acree and Kier models. This hypothesis has not found any further confirmation in the literature. NMR studies (Dick, 1981) on flavans and flavanones related to phyllodulcin demonstrated a preferred semiplanar conformation in solution for these compounds, which fits better with the flat cavity described by Temussi and coworkers. A similar conclusion has been drawn also by us in a preceding paper (Arnoldi et al., 1991), where we studied the preferred conformations of oxygenated isovanillyl derivatives, which generally have a chair or half-chair conformation with the isovanillyl substituent in a pseudoequatorial position. In that paper, we also faced another problem, trying to establish which is the angle between the two planes defined by the two aromatic rings (β). In the minimum energy conformation we found that β was generally comprised between 60 and 90 degrees; however, the energy barrier to the rotation was very low ($\Delta E < 2$ kcal), thus leaving open the question of the actual position of ring C in the active conformation.

Therefore we started the synthesis of rigid derivatives with the aim to establish whether a preferred conformation for the hydrophobic moiety in isovanillyl derivatives could be recognized. In these derivatives, the aromatic or aliphatic ring C is blocked in order to be almost planar (compounds **3**–**5**) or almost perpendicular (compounds **6**–**8**) with respect to ring A.

The seven rigid isovanillyl derivatives described in this paper are shown in Figure 3.

The new rigid derivatives were tasted and compared with some flexible analogues, shown in Figure 4. The minimum energy conformations were calculated by molecular modeling (see Materials and Methods). The solid state conformation of compound (±)-**14** was determined by X-ray analysis.

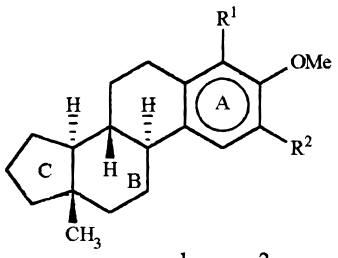
The relationships between conformation and taste were studied by comparison with the current models of sweet taste receptor. Data analysis was performed by principal components analysis (PCA), factor and cluster analysis, looking for relationships between geometrical descriptors of the compounds and sweet taste.

MATERIALS AND METHODS

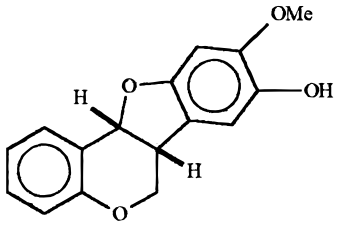
General Procedures. Melting points are uncorrected. Flash chromatographies were done on silica gel Merck (230–400 mesh); NMR spectra were recorded on Bruker WP80 at 80 MHz and Varian XL300 instruments using tetramethylsilane as internal standard; chemical shifts are expressed in ppm; *J* values are in Hz. MS spectra were recorded on a Finnigan TSQ70 spectrometer equipped with an ICIS data system. IR spectra were recorded with a Perkin-Elmer 1310 infrared spectrophotometer. Solvents and reagents were of analytical grade and were purchased from Sigma-Aldrich, Milwaukee, U.S.A.

The listed compounds were synthesized following literature methods: 3-hydroxy-1,3,5-estratriene (Huang-Minlon, 1949), 3-methoxy-1,3,5-estratriene (Sax et al., 1964), 5-hydroxy-6-methoxyindan-1-one (**21**), (Cannon et al., 1990), 5-methoxy-6-hydroxyindan-1-one (**20**) (Koneck and Szamack, 1922), 4-hydroxy-5-methoxyindan-1-one (**24**) (Fujii et al., 1977), substituted pterocarpan **5** (Engler et al., 1990), 2-mercaptomethylbenzenethiol (Arnoldi and Carughi, 1988). The synthesis of compounds **10**, **11**, **13**–**16** is described in preceding papers (Arnoldi et al., 1986, 1991).

2-Formyl-3-methoxy-1,3,5-estratriene (18) and 4-Formyl-3-methoxy-1,3,5-estratriene (19). In a dry flask under nitrogen 3-methoxy-1,3,5-estratriene (1.4 g, 5 mmol) was



	R^1	R^2
3	OH	H
4	H	OH



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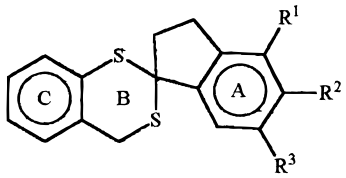
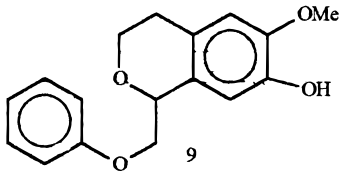


	R^1	R^2	R^3
6	H	OMe	OH
7	H	OH	OMe
8	OH	OMe	H

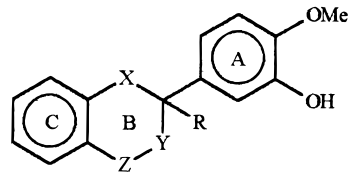


9

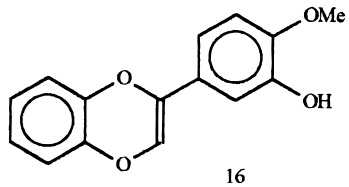
cpd.	Relative Sweetness
3	tasteless
4	sweet, 50x
5	tasteless
6	sweet, 50x
7	tasteless
8	tasteless
9	tasteless

Figure 3. Structure and relative sweetness (RS, calculated by comparison with an aqueous 3% sucrose solution, see Materials and Methods) of compounds **3**−**9**.

dissolved in THF and cooled at −78 °C. Two milligrams of 2,2′-dipyridyl was added as an indicator and sec-BuLi (1 mL, 1M solution in THF) was added dropwise until the red color persisted, followed by further 8.7 mL of the same reagent. After 2.5 h dry *N*,*N*-dimethylformamide was added (3.5 mL, 5 mmol), and the reaction mixture was allowed to reach room temperature. Ice (70 g) and HCl (3 N, 8 mL) were added



	X	Y	Z	R
10	CH_2	CH_2	O	H
11	O	CH_2	O	H
12	O	CH_2	O	CH_3
13	O	O	CH_2	H
14	S	O	CH_2	H
15	S	S	CH_2	H



16

cpd.	Relative Sweetness
10	sweet, 100x
11	sweet, 450x
12	tasteless
13	sweet, 3000x
14	*R*-(+): sweet, 18000x; *S*-(-): tasteless
15	*R*-(+): sweet, 20000x, *S*-(-): tasteless
16	tasteless

Figure 4. Structure and relative sweetness (RS) of compounds **10**−**16**.

keeping the temperature lower than 5 °C. The aqueous phase was extracted with ethyl acetate, dried over sodium sulfate, evaporated to dryness, and chromatographed (hexanes−ethyl acetate 9:1) to give **18** 0.3 g, (20%) and **19** 0.1 g, (7%).

18: mp 148 °C; ^{1}H NMR ($CDCl_3$), δ: 0.75 (s, 3H, Me), 1.0−2.3 (m, 15H), 2.8−3.1 (m, 2H), 3.9 (s, 3H, OMe), 6.7 (s, 1H, ar), 7.75 (s, 1H, ar) 10.62 (s, 1H, CHO); MS *m*/*z* (%): 298 (38), 163 (38), 128 (20); IR 1670 cm^{-1}; $[\alpha]_D$ = +90.22 ($CHCl_3$, *c* 0.266). Anal. Calcd for $C_{20}H_{26}O_2$: C, 80.46; H, 8.78. Found: C, 80.39; H, 9.00.

19: mp 164 °C; ^{1}H NMR ($CDCl_3$), δ: 0.75 (s, 3H, Me), 1.0−2.3 (m, 15H), 3.1−3.2 (m, 2H), 3.9 (s, 3H, OMe), 6.8 (d, J = 9, 1H, ar), 7.5 (d, J = 9, 1H, ar), 10.68 (s, 1H, CHO); MS *m*/*z* (%): 298 (100), 187 (10); IR 1670 cm^{-1}; $[\alpha]_D$ = +100.66 ($CHCl_3$, *c* 1.2). Anal. Calcd for $C_{20}H_{26}O_2$: C, 80.46; H, 8.78. Found: C, 80.46; H, 8.43.

3-Methoxy-1,3,5-estratrien-2-ol (3). Compound **18** (0.3 g, 1 mmol) was dissolved in dry dichloromethane and refluxed for 2 h with 85% MCPBA (0.2 g, 1 mmol). The reaction mixture was evaporated, dissolved in ethyl acetate, washed with saturated $NaHCO_3$, dried, and evaporated to dryness. The residue was dissolved in methanol and stirred under nitrogen for 5 h with 5% KOH (1 mL). The reaction was concentrated, acidified with HCl, exctracted with ethyl acetate, and evaporated. The residue was chromatographed (hexanes−ethyl acetate 8:2 v\v) to give **3** as a white solid (0.12 g, 42%). Mp (aqueous ethanol) 123 °C; ^{1}H NMR ($CDCl_3$) δ: 0.75 (s, 3H, Me), 0.85−2.9 (m, 17H), 3.84 (s, 3H, OMe), 5.36 (s, 1H, OH), 6.58 (s, 1H, ar), 6.90 (s, 1H, ar); MS *m*/*z* (%): 286 (100), 189 (5.5), 137 (5), 95 (4); $[\alpha]_D$ = +6.67 ($CHCl_3$, *c* 0.42). Anal. Calcd for $C_{19}H_{26}O_2$: C, 79.68; H, 9.15. Found: C, 78.76; H, 9.05.

3-Methoxy-1,3,5-estratrien-4-ol (4). Obtained from **19** with a similar procedure. Chromatography gave a white solid, 42.5 mg, 64%. Mp (aqueous ethanol) 117 °C. ^{1}H NMR ($CDCl_3$), δ: 0.75 (s, 3H, Me), 0.90−2.95 (m, 17H), 3.84 (s, 3H, OMe), 5.65 (s, 1H, OH), 6.71−6.83 (dd, J = 8.28, 2H, ar); MS *m/z* (%): 286 (100), 189 (5.8), 95 (7); $[\alpha]_D$ = +71.9 ($CHCl_3$, *c* 0.63). Anal. Calcd for $C_{19}H_{26}O_2$: C, 79.68; H, 9.15. Found: C, 79.98; H, 9.16.

5-(2,2-Dimethylpropanoyloxy)-6-methoxyindan-1-one (22). 5-Hydroxy-6-methoxyindan-1-one (1.22 g, 7 mmol) was dissolved in dry dichloromethane. Pyridine (1 mL, 14 mmol), 2-pyrrolidinopyridine (0.1 mL), and pivaloyl chloride (0.9 mL, 7.2 mmol) were added. After refluxing for 12 h, the solution was poured in water and acidified, the organic phase was separated, washed with brine, dried over sodium sulfate, and evaporated to dryness. After chromatography (hexanes−ethyl acetate 65:35 v/v) compound **22** was obtained as a white solid (1.4 g, 76%). Mp 107 °C. ^{1}H NMR ($CDCl_3$), δ: 1.36 (s, 9H, t-Bu), 2.70 (m, 2H, H-3), 3.15 (m, 2H, H-2), 3.82 (s, 3H, OMe), 7.10−7.25 (s, 2H, H-4 and 7); MS *m/z* (%): 262 (29), 178 (100), 135 (2), 85 (4).

Spiro[(4-H-1,3-benzodithian)-2,1′-(5′-(2,2-dimethylpropanoyloxy)-6′-methoxy-[1*H*]-2′,3′-dihydroindene)] (23). A solution of 2-mercaptomethylbenzenethiol (185 mg, 1.2 mmol) and the substituted indanone **22** (300 mg, 1.2 mmol) in CH_2Cl_2 was cooled at 0 °C, saturated with HCl, and stirred for 48 h. The reaction mixture was diluted with water, and the organic phase was separated, washed with aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated to dryness. Chromatography (hexanes−ethyl acetate 9:1 v/v) gave **23** as a white solid (460 mg, 98%). ^{1}H NMR ($CDCl_3$), δ: 1.4 (s, 9H, t-Bu), 2.5 (m, 2H, H-2′(3′)), 2.95 (m, 2H, H-3′(2′)), 3.8 (s, 3H, OMe), 4.0 (s, 2H, H-4), 5.6 (s, 1H, OH), 6.7 and 6.8 (two s, 2 × 1H, H-4′ and 7′), 7.3 (4H, m, ar); MS *m/z* (%): 400 (58), 367 (100), 283 (67), 194 (20), 105 (23).

Spiro[(4-H-1,3-benzodithian)-2,1′-(5′-methoxy-6′-hydroxy-[1*H*]-2′,3′-dihydroindene)] (6). A mixture of 2-mercaptomethylbenzenethiol (110 mg, 0.7 mmol) and 5-methoxy-6-hydroxyindan-1-one (100 mg, 0.56 mmol) was dissolved in 50 mL of dichloromethane, cooled at 0 °C, and saturated with HCl gas. After stirring for 48 h, the mixture was poured in water, and the organic phase was separated, washed with $NaHCO_3$ and brine, dried over Na_2SO_4 and evaporated to dryness to give a red oil which was purified by column chromatography (hexanes−ethyl acetate 8:2 v/v) to give **6** as a yellowish low-melting solid. ^{1}H NMR ($CDCl_3$), δ: 2.5 (m, 2H, *CH*$_2$-*3′*), 2.95 (m, 2H, *CH*$_2$-*2′*), 3.87 (s, 3H, OMe), 3.95 (dd, 2H, CH_2S), 5.5 (s, 1H, OH), 6.7 and 6.85 (s 2H, ar), 7.3 (m, 4H, ar); MS *m/z* (%): 316 (42), 283 (100), 194 (39), 193 (34), 122 (22), 121 (43).

Spiro[(4-H-1,3-benzodithian)-2,1′-(5′-hydroxy-6′-methoxy-[1*H*]-2′,3′-dihydroindene)] (7). $LiAlH_4$ (53 mg, 1.4 mmol) was suspended in anhydrous THF. A solution of **23** (0.46 g, 1.1 mmol) in THF was added dropwise and the solution was stirred for 36 h at room temperature. Ethyl acetate (2 mL) was added followed by aqueous HCl. The acid solution was exctracted with ethyl acetate, washed with brine, dried over sodium sulfate, and evaporated to dryness to give a white solid (120 mg, 27%). Mp 137 °C (ethyl acetate); ^{1}H NMR ($CDCl_3$), δ: 2.55 (m, 2H, H-2′(3′)), 2.95 (m, 2H, H-3′(2′)), 3.80 (s, 3H, OMe), 4.00 (s, 2H, H-4), 5.62 (s, 1H, OH), 6.70 and 6.85 (two s, 2 × 1H, H-4′ and 7′), 7.20−7.40 (m, 4H, ar); MS *m/z* (%): 316 (40), 283 (100), 194 (39), 193 (24), 121 (43). Anal. Calcd for $C_{17}H_{16}O_2S_2$: C, 64.55; H, 5.06. Found: C, 63.97; H, 4.91.

Spiro[(4-H-1,3-benzodithian)-2,1′-(4′-hydroxy-5′-methoxy-[1*H*]-2′,3′-dihydroindene)] (8). A solution of 2-mercaptomethylbenzenethiol (100 mg, 0.56 mmol) and the substituted indanone **24** (110 mg, 0.67 mmol) in CH_2Cl_2 was cooled at 0 °C, saturated with HCl, and stirred for 48 h. The reaction mixture was diluted with water, and the organic phase was separated, washed with aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated to dryness. Chromatography (hexanes−ethyl acetate 8:2 v/v) gave **8** as a yellow oil (120 mg, 68%). MS *m/z* (%) 316 (32), 284 (21), 283 (100), 251 (24), 223 (14), 194 (56), 193 (37), 179 (18), 161 (68); ^{1}H NMR ($CDCl_3$), δ: 2.60 (m, 2H, H-2′), 3.00 (m, 2H, H-3′), 3.90 (s, 3H, OMe), 4.00 (s, 2H, H-4), 5.65 (s, 1H, OH), 6.78−7.40 (m, 6H, ar). Anal. Calcd for $C_{17}H_{16}O_2S_2$: C, 64.55; H, 5.06. Found: C, 64.07; H, 5.25.

2-Phenoxymethyl-2*H*-7,8-dihydrobenzopyran (9). A solution of homovanillyl alcohol (500 mg, 3 mmol) and phenoxyacetaldehyde dimethylacetal (0.5 mL, 3 mmol) in dry THF was saturated with HCl. The reaction mixture was stirred overnight, evaporated to dryness, and extracted with ethyl acetate. The organic phase was washed with aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated to give a yellow oil which was chromatographed on silica gel (hexanes−ethyl acetate 8:2 v/v) to give **9** as white needles (520 mg, 60%). Mp 96 °C (EtOH−water 1:1); ^{1}H NMR ($CDCl_3$), δ: 2.60−2.75 (m, 1H, H-7a(b)); 2.85−3.00 (m, 1H, H-7b(a)); 3.75−3.85 (m, 1H, H-8a(b)); 3.90 (s, 3H, OMe); 4.15−4.30 (m, 3H, CH_2OPh and H-8b(a)), 5.10 (m, 1H, H-2); 6.65 and 6.80 (two s, 2 × 1H, H-3 and H-6), 6.95−7.00 (m, 3H, ar); 7.20−7.35 (m, 2H, ar); MS *m/z* (%): 286 (10), 179 (100), 91 (10), 44 (10), 32 (40). Anal. Calcd for $C_{17}H_{18}O_4$: C, 71.31; H, 6.52. Found: C, 71.22; H, 7.01.).

2-Methyl-2-(3-hydroxy-4-methoxyphenyl)-1,4-dihydrobenzodioxane (12). A solution of 1-(3-hydroxy-4-methoxyphenyl)-2-(2-hydroxyphenoxyethanone) (Arnoldi et al., 1986) (0.2 g, 0.73 mmol) in 15 mL of dry toluene was added with 0.73 mL (2.19 mmol) of methylmagnesium bromide (3 M in THF) and left 1.5 h at room temperature. The reaction mixture was poured into a mixture of ice and concentrated HCl and extracted with ethyl acetate. 1-(2-Hydroxyphenoxy)-2-(3-hydroxy-4-methoxyphenyl)propanol (0.16 g) was obtained by column chromatography with methylene chloride−ethyl acetate 7:3 v/v as an oil [MS *m/z* (%) 272 (58), 167 (100), 137 (36), 109 (14); ^{1}H NMR ($CDCl_3$), δ: 1.65 (3H, s, CH_3), 2.55 (1H, OH), 3.9 (3H, s, OCH_3), 4.1 (2H, s, CH_2), 5.65 (1H, OH), 6.0 (1H, OH), 6.8−7.1 (7H, m, ar)] and used without further purification. This compound (0.12 g, 0.41 mmol) was dissolved in 10 mL of toluene, added with 0.06 g of Amberlyst 15 ion exchanger, and refluxed for 30 min. Filtration, evaporation, and chromatography with hexanes−ethyl acetate 7:3 v/v gave 100 mg of (**12**), as an oil. MS *m/z* (%) 272 (100), 211 (8), 164 (66), 149 (42), 121 (22), 103 (18), ^{1}H NMR ($CDCl_3$), δ: 1.60 (3H, s, CH_3), 3.9 (3H, s, OCH_3), 4.1 (2H, dd, CH_2), 5.6 (1H, s, OH), 6.8−7.1 (7H, ar). Anal. Calcd for $C_{16}H_{16}O_4$: C, 70.57, H, 5.92. Found: C, 70.21, H, 6.06.

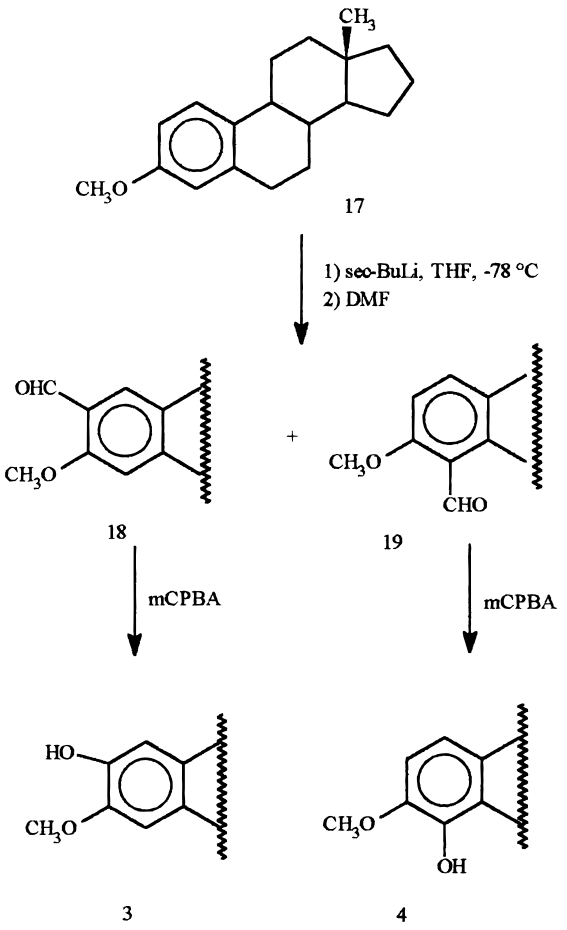
Crystal Structure of Compound (±)-14. The compound was crystallized by slow diffusion of hexane in a benzene solution. Crystals were monoclinic, centrosymmetric space-group *C*2/*C*, a = 25.992(12), b = 7.448(5), c = 15.820(7) Å, β = 121.83(1)°, U = 2602 Å^3, Z = 8; 1687 unique reflections were collected on a MARresearch Image Plate System and the structure was refined to R = 0.0590. Full details have been deposited at the Cambridge Crystallographic Data Centre.

Conformational Analysis. The molecular models for all the considered compounds were built on a Silicon Graphics IRIS 35, using the program INSIGHT II, 95.0 (Molecular Simulation Inc., San Diego, CA). The initial models were refined by molecular mechanics techniques: the DISCOVER program (Molecular Simulation Inc., San Diego, CA) was used to generate low-energy conformations on which we measured (directly or deriving them) the geometrical descriptors utilized in the principal components analysis (PCA).

Statistical Analysis. Principal components analysis (PCA), factor analysis, and cluster analysis were performed with the program SYSTAT 5.0 (Systat Inc.).

Tasting. All the compounds assayed in this work were tasted only once with the "sip and spit" procedure. 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.

Scheme 1



The relative sweetness, RS, is defined as RS = [sucrose]/[sweetener]$_{isosweet}$. The reported Relative Sweetness values are referred to racemic compounds, unless specified. The precision of the values could not be assessed, as the compounds were tasted only once, because of safety precautions.

RESULTS AND DISCUSSION

Synthesis of Rigid Compounds. For the synthesis of the planar compounds **3** and **4** we used as the starting compound estrone, which is commercially available in optically active form (Scheme 1).

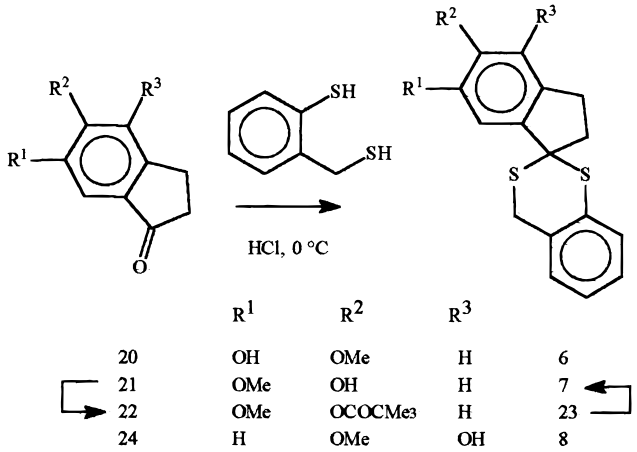
In the first step, the keto group of estrone was removed following a literature method (Huang-Minlon, 1949). After methylation of the phenolic group with dimethyl sulfate, the methyl ether **17** was lithiated in the *ortho* positions with sec-BuLi at −78 °C and formylated with *N*,*N*-dimethylformamide (Perth and Ridley, 1989) to give a 4:1 mixture of the aldehydes **18** and **19** that were separated by flash chromatography. Baeyer−Villiger oxidation with 3-chloroperbenzoic acid (MCPBA), followed by basic hydrolysis of the intermediate formic esters, gave the optically active isomeric derivatives **3** and **4**.

The substituted pterocarpan **5** was synthesized as described by Engler et al. (1990).

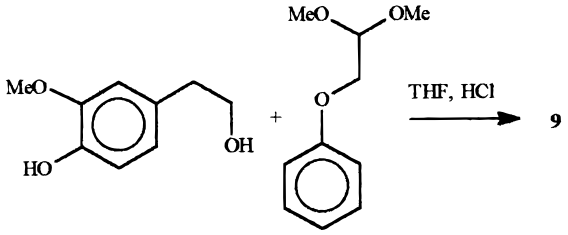
The spiranic derivatives **6**−**8** were obtained by cyclization of 2-mercaptomethylbenzenethiol (Arnoldi and Carughi, 1988) with a substituted indan-1-one (Scheme 2).

The synthesis of 5-methoxy-6-hydroxyindan-1-one **20** described in the literature (Koneck and Szamack, 1922) was slightly modified in order to improve the yield.

Scheme 2



Scheme 3



Ferulic acid was hydrogenated to dihydroferulic acid and converted to its acyl chloride, which was submitted to intramolecular Friedel−Crafts acylation with $AlCl_3$ to give **20**. This was reacted with 2-mercaptomethylbenzenethiol in acid conditions to give **6**.

A similar strategy was used for the synthesis of 5-hydroxy-4-methoxyindan-1-one **21**, starting from 3-hydroxy-4-methoxyphenylpropionic acid (Rosazza et al., 1970). In this case, the thioketal **7** could not be obtained directly from **21**, but the previous protection of the phenolic hydroxy group with an electron withdrawing group was required. Thus **21** was esterified with pivaloyl chloride, and the ester **22** cyclized to give the derivative **23** which gave **7** by deprotection with $LiAlH_4$.

For the synthesis of indanone **24**, we first prepared 4,5-dimethoxyindanone (Ahmad and Snieckus, 1982) that was demethylated selectively (Fujii et al., 1977) to give **24**. This was reacted with 2-mercaptomethylbenzenethiol to give the isovanillyl derivative **8**.

The reactivity of these substituted indanones in ketalization reactions was generally low: they did not react with salicylic alcohol or 2-mercaptobenzyl alcohol either directly or by transketalization with the corresponding dimethylketals, with different acid catalysts. Besides the strong nucleophilic 2-mercaptomethylbenzenethiol, only 1,3-dimercaptopropane was able to react with **21** to give the corresponding cyclic thioketal.

The tricyclic derivative **9** was synthesized in one step and 60% yield from homovanillyl alcohol and phenoxyacetaldehyde dimethylacetal (Scheme 3).

The new derivatives were tasted (see Tasting) in order to assess their taste and, if sweet, the relative sweetness (RS) (Figures 1 and 2).

Structure−Taste Relationships. The most important observation derived from the tasting trials is that the rigid isovanillyl derivatives were tasteless or much less sweet than their flexible analogues. This could probably be attributed to several effects, such as the lacking of the heteroatoms in some cases, their incorrect

position, and also the steric hindrance introduced by the new ring. This difference holds also in the case of compounds **6**, **8**, and **15**, characterized by a high structural similarity, but whose relative sweetness is very different, the flexible compound **15** being by far the sweetest of the whole series, while **6** has a Relative Sweetness of 50× and **8** is tasteless. Another point is that the sweet taste is retained in both a flat (**4**) and a spiranic (**6**) derivative, thus showing that there should be a certain tolerance for the position of rings B and C around the *z* axis.

On the other hand, also compound **9**, which has the greatest conformational flexibility for what concerns ring C, is completely tasteless. In this compound, structurally similar to compound **11** (Relative Sweetness = 450×), only the oxygen atom in ring B has been blocked in an almost coplanar position with respect to ring A.

The overall impression was that the geometrical features of the derivatives, beside their structural characteristics, seemed to play a very important role in the interaction with the receptor.

As a first approach, starting from the simple hypothesis of a three-site interaction with the receptor, these rigid derivatives were compared with the models by Temussi and Goodman (Temussi et al., 1978, 1991; Ciajolo et al., 1983; Douglas and Goodman, 1991; Yamazaki et al., 1991, 1994) to see whether they fitted the primary topological requirements of the models.

However, in the case of rigid isovanillyl derivatives, this comparison did not give any conclusive information. Indeed, compounds **4** (planar) and **6** and **8** (spiranic) seem to fit better the Goodman model, while compounds **4** and **8** seem to fit better the Temussi model. Nothing is possible to say about compound **9**, owing to its greater flexibility. Only compound **7** is clearly noncompatible with these models because of the wrong AH-B-G relative positions. Nevertheless, direct comparison of different classes of compounds between each other and with these models is not always easy, because each author uses a different reference system for the Cartesian coordinates. Therefore, only a qualitative comparison can be made.

By simple comparison with these models we could neither explain the tasting results nor answer the question about the actual position(s) adopted by B and C rings with respect to the isovanillyl moiety in the biologically active conformation. Another limitation of this approach (to use rigid compounds as molecular molds) is that the synthesis of rigid compounds is quite time-consuming, and the geometrical features of the target molecule could be planned only partially.

A very important question to be considered is the role of configuration. Among isovanillyl sweet compounds, *R*-(+)-phyllodulcin is sweet, while the *S*-(−)-enantiomer is tasteless (Zehnter and Gerlach, 1996). We were able to separate and taste two other couples of enantiomeric derivatives, **14** and **15,** and to assign their absolute configuration (unpublished results). Also in this case, the *R* enantiomers (which are sterically related to *R*-(+)-phyllodulcin) resulted sweet, while the *S* enantiomers were tasteless. This is in accordance with the fact that the receptor cavity is asymmetrical. In other words, both enantiomers of every compound should be compared independently with the model, their interaction being diastereotopic.

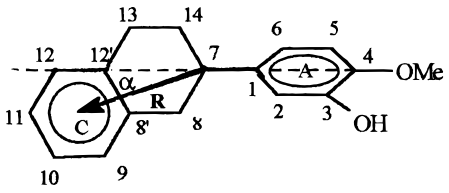


Figure 5. Geometrical descriptors used in the principal components analysis of isovanillyl derivatives: distance R, the module of the vector lying between the center of ring C and C7 (Å); angle α, the angle between the vector R and the axis of atoms C1−C4 of the isovanillyl group; angle β, the angle between the plane of ring C and the plane of ring A; the dihedral angles δ: δ_1 = 2-1-7-8; δ_2 = 1-7-8-8′; δ_3 = 1-7-14-13; δ_4 = 7-14-13-12′.

Table 1. Geometrical Parameter Values of the Compounds Analyzed by PCA

compd	α	*R*	δ_1	δ_2	δ_3	δ_4	β
R1	23.67	3.82	−78.30	171.22	−169.72	18.44	108
S1	23.67	3.82	78.30	−171.22	169.72	−18.44	252
3	−22.87	3.69	164.29	174.62	−179.31	−51.61	180
4	22.87	3.69	−21.67	174.59	−179.34	−51.63	180
RR5	−43.58	3.80	49.22	133.66	−110.07	−37.27	215
SS5	−43.58	3.80	−49.22	−133.66	110.07	37.27	145
R6	68.86	4.07	−50.41	89.32	−133.84	53.52	68
S6	68.88	4.07	50.42	−89.29	133.80	−53.49	292
R8	−68.12	4.07	130.86	90.39	−135.11	54.36	293
S8	−68.09	4.07	−130.86	−90.44	135.17	−54.41	67
R10	22.93	3.85	−64.31	172.43	−179.66	37.19	89
S10	22.93	3.85	64.31	−172.43	179.66	−37.19	271
R11	32.2	3.77	−46.45	162.04	−171.10	37.57	68
S11	32.2	3.77	46.45	−162.04	170.93	−37.57	292
R12	37.56	3.81	−1.65	152.07	−165.91	40.29	213
S12	37.56	3.81	1.65	−152.07	165.91	−40.29	147
R13	37.38	3.77	−60.13	153.94	−173.84	46.88	78
S13	37.38	3.77	60.13	−153.94	173.84	−46.88	282
R14	31.33	4.09	−43.19	150.99	173.84	64.77	62
S14	31.33	4.09	43.19	−150.99	−173.84	−64.77	298
R15	25.83	4.18	−60.14	168.61	170.93	61.69	74
S15	25.83	4.18	60.14	−168.61	−170.93	−61.69	286
16	−22.52	3.74	−116.33	−179.00	178.94	−0.45	116

As the simple comparison of our compounds with the known models could not explain the taste results, we decided to perform a structure−activity relationship study on this class of compounds.

Since the aim of this work was to find the relationships between conformation and taste, taking also into account the stereochemistry, we considered only geometrical descriptors and analyzed them by statistical tools, such as principal components analysis, factor analysis, and cluster analysis. Figure 5 represents the generic structure of an isovanillyl compound to which all the compounds of this study can be referred, together with the description of the geometrical parameters utilized.

It can be noticed that α and R are identical for the two enantiomers of any compound; δ (*R*) and δ (*S*) are opposite, while $\beta(R) + \beta(S) = 360°$. α and R, already defined and utilized in a previous paper of our group (Arnoldi et al., 1991), are independent from the rotation around the pivot bond C7−C1.

Table 1 lists the compounds and the values of the geometrical descriptors utilized in our structure−activity relationship study and analyzed by PCA.

The component loadings and the percent of total variance explained by the first four principal components are shown in Table 2.

The loadings of factor 1 are dominated by β and δ_1, which depend on the position of ring C with respect to ring A. The highest component loadings for factor 2

Table 2. Component Loadings and Percent of Total Variance Explained

	factor			
	1	2	3	4
β	−0.933	−0.176	0.063	0.040
δ_1	−0.895	0.161	0.031	−0.036
δ_4	0.684	0.388	0.248	0.019
δ_2	0.395	0.851	−0.030	−0.001
δ_3	0.119	−0.892	0.063	−0.011
R	0.013	−0.068	0.981	0.036
α	0.006	0.010	0.036	0.999
% of total variance explained	32.99	24.73	14.78	14.32

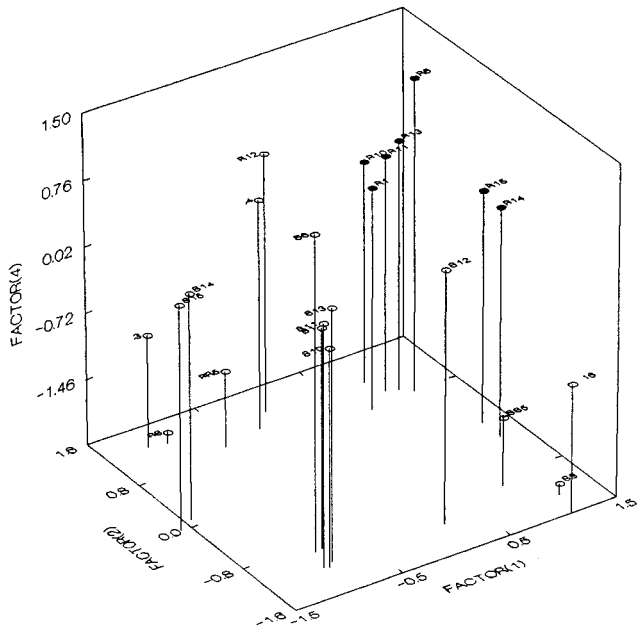


Figure 6. Score plot of factors 1, 2, and 4 for the compounds listed in Table 1. The sweet derivatives are indicated by solid dots.

correspond to δ_3 and δ_2, parameters strictly related to the conformation of the heterocycle B. The sum of these two factors accounts for about 58% of the total variance. α and R give their major contribution to factors 3 and 4, which are able to explain only 14.78 and 14.32% of the total variance, respectively. This is consistent with the fact that they are identical for enantiomeric compounds.

Figure 6 shows the score plot of factors 1, 2, and 4 for the compounds submitted to statistical analysis (a little less apparent clustering is obtained by plotting factors 1−3).

The sweet compounds *R*-**1**, *R*-**14**, and *R*-**15** are clustered in a confined region of the chart, together with the *R* enantiomers of the other sweet compounds tasted as racemates (*R*-**6**, *R*-**10**, *R*-**11**, and *R*-**13**), which are therefore predicted to be sweet. Remarkably, their *S* enantiomers, with the other tasteless compounds, are all located outside this cluster.

It is important to notice how excluding α and R from the data set the cluster of sweet compounds was not obtained: this means that these parameters are not negligible.

The factor scores obtained by PCA were analyzed by cluster analysis, using the minimum Euclidean distance algorithm. The hierarchical tree plot obtained is shown in Figure 7. An analogous hierarchical tree was obtained by cluster analysis directly applied to the data matrix represented in Table 1.

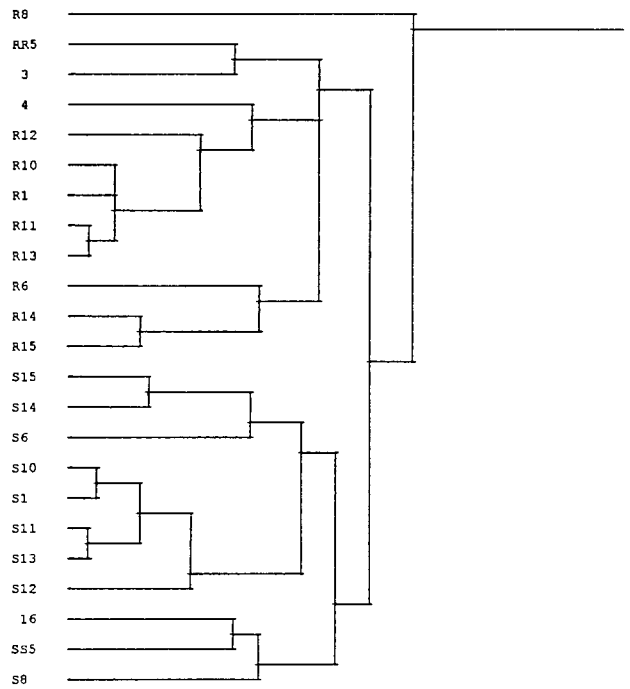


Figure 7. Hierarchical tree plot obtained by cluster anlysis (single linkage method, nearest neighbor).

Again compounds *R*-**1**, **4**, *R*-**6**, *R*-**10**, *R*-**11**, and *R*-**13** are joined in a cluster. It is interesting to notice how by this objective procedure of clustering, the obtained cluster includes also compound **4**, which appears as an outlier in the tridimensional plot of Figure 4. On the other hand, also the tasteless compound *R*-**12** is included in this cluster. A second cluster contains compounds *R*-**14** and *R*-**15**, which are the sweetest of the series, with a taste potency of 1 order of magnitude greater than the other sweet compounds.

From the statistical analysis applied to isovanillyl derivatives some important conclusions can be drawn. First of all the use of simple geometrical parameters on this homogeneous set of compounds led to a description of the relationships between taste and conformation. The same result could not be obtained only from the comparison of rigid derivatives with the existing models.

Figure 8 shows the minimum energy conformations of the compounds object of this study, superimposed by their isovanillyl ring A.

For the sweet compounds (Figure 8b) the hydrophobic moiety can occupy only a limited region of space; the hydrophobic ring C lies mostly in a plane almost perpendicular to that of the isovanillyl ring A but also a semiplanar position is allowed. Thus, it seems likely that for this class of sweet compounds the space available along the *z* axis for the hydrophobic site G in the receptor cavity is large enough to include a perpendicular aromatic ring. This conformation is rather different from the flat one suggested as the active conformation in previous hypotheses by DuBois and colleagues (1977), Dick (1981) and accepted also by us (Arnoldi et al., 1991) in a preceding work but is consistent with the existence of very large hydrophobic groups (e.g. cyclononane) such as those found in the very sweet guanidinic derivatives (Tinti et al., 1991).

It can also be noticed (Figure 8) that for the sweet derivatives there are two small zones where the heteroatoms fall and are almost superimposed, whereas in

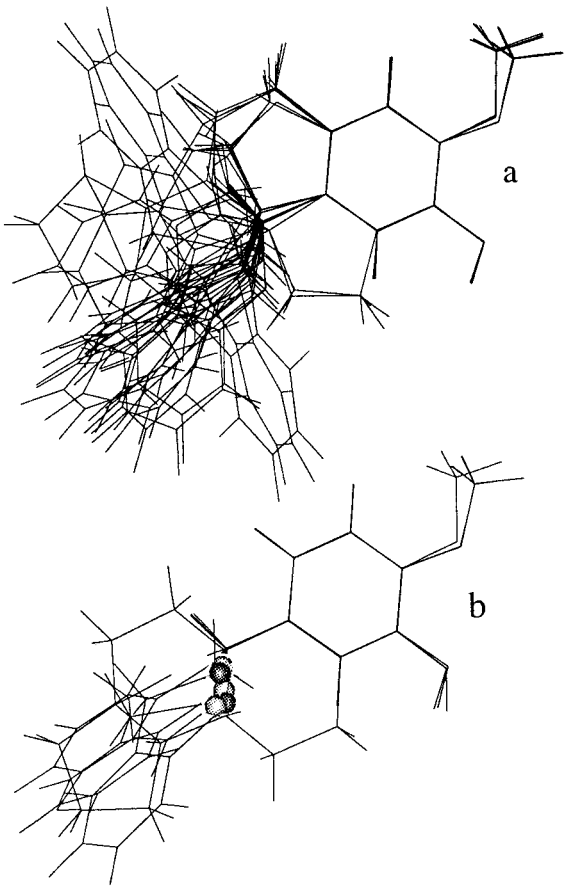


Figure 8. Superimposition of the minimum energy conformations of the compounds listed in Table 1: (a) all the compounds and (b) only sweet derivatives. The heteroatoms are shown as solid dots.

tasteless compounds their positions are distributed widely apart. This suggests that the position of ring B, and in particular of the heteroatoms, is important to establish a positive interaction of the molecules with the receptor. In fact, the importance of heteroatom type, number, and position in isovanillyl derivatives has been already noticed by us (Arnoldi et al., 1991, 1993, 1996) even if a rational explanation of this effect could not be found. Thus, the existence of a new interaction site, different from the pure hydrophobic G site, and which is specifically involved in a bond with the heteroatoms, could perhaps be suggested; both the effects could contribute to the overall interaction with the receptor. This hypothesis could also explain the sweet taste of the spiranic derivative **6**, which is well superimposed with *R*-**15** by means of its ring B, whereas ring C appears staggered out. Likewise, compound **9** could be tasteless because the oxygen atom in the heterocyclic ring is coplanar with ring A, while any position can be occupied by ring C (due to its great flexibility).

Although the results obtained appear satisfactory in terms of analysis of the structural features of the compounds it must however be observed in general that these studies do not take account of the natural medium of interaction between the sweet compounds and the receptor, i.e., water. This task is however of great difficulty, and only recently work along this line has begun (Astley et al., 1996; Mathlouthi, 1996).

Noteworthily, the calculated conformation for sweet flexible compounds is similar to that assumed in the solid state by compound **14**, as determined by X-ray

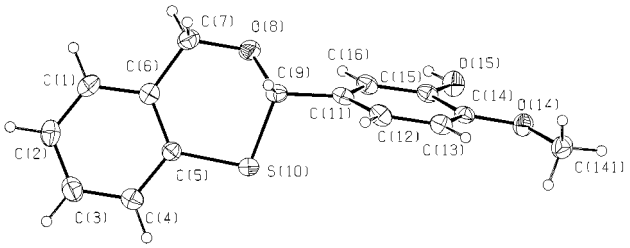


Figure 9. Crystal structure of compound (±)-**14** with the thermal ellipsoids showing 30% occupancy.

analysis (Figure 9). It is known from the literature (Böhm, 1996) that small hydrophobic molecules often assume in the active site of proteic macromolecules a conformation very similar to that of the solid state.

The second important feature of the statistical analysis applied to rigid compounds is that it permits distinction between the sweet and the tasteless enantiomer of the same compound. We suppose that the sweet taste is due to only one of the enantiomers also for the compounds tasted as racemates, the sweet enantiomer being the one stereochemically related to *R*-(+)-phyllodulcin. Adding other parameters, such as electronic or lipophilic, this difference is reduced, because they are identical for the two enantiomers.

As in isovanillyl derivatives the difference in taste of the two enantiomers seems to be general and could help in defining the receptor active site, further studies on relationships between configuration and taste are currently under investigation in our group.

ABBREVIATIONS USED

NHDC, neohesperidin dihydrochalcone; MCPBA, 3-chloroperbenzoic acid.

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