Isovanillyl Sweeteners. Synthesis, Conformational Analysis, and Structure–Activity Relationship of Some Sweet Oxygen Heterocycles

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New benzodioxin, benzodioxole, benzodioxepine and isoflavan analogues of the natural isovanillyl sweetener phyllodulcin have been synthesized and their sweetness assessed. Molecular-mechanics calculations on 15 members of the class of the isovanillyl sweet compounds indicated a preferential quasi-orthogonal orientation of the isovanillyl ring with respect to the heterocyclic ring. Experimental confirmation was obtained by a detailed study of transient NOE (nuclear Overhauser effect) experiments on 2-(3-hydroxy-4-methoxyphenyl)-1,4-benzodioxane. A theoretical treatment of these experiments is given. As, however, the energy barrier for the rotation of the isovanillyl ring around the bond linking it to the heterocyclic ring is low, geometrical parameters independent of this rotation were sought and an empirical correlation with sweetness potency established. The results are discussed in terms of the current hypotheses on the receptor shape.

Isovanillyl sweeteners are a class of sweet compounds, the first representative of which was phyllodulcin **1**, a natural dihydroisocoumarin[1] isolated from the leaves of *Hydrangea serrata* SERINGE var. *thunbergii* SUBIMOTO and used in Japan for the preparation of a sweet infusion.

Other substances of natural origin (Fig. 1) which are structurally related to **1** and have sweet potency are neohesperidin dihydrochalcone **2**,[2] a semisynthetic glucoside obtained by alkali-catalysed ring-fission and hydrogenation of the corresponding bitter flavanone glucoside which is extracted from orange peel, and dihydroquercetin3-acetate 4'-(methylether) **3**, obtained by modification of a natural 3-hydroxyflavan-4-one recently isolated from *Tessaria dodoneifolia*.[3] The potency of the sweet taste of these three compounds is, respectively, 400[4] or 600–800[5] for phyllodulcin, 665[2a] or 340–390[2b,c] for neohesperidin dihydrochalcone, and 400 for compound **3**[3] with respect to sucrose on a weight basis.

It was suggested by Shallenberger and Acree[6] and by Kier[7] and it is now generally accepted[8] that the sweet taste of these compounds is due to the 3-hydroxy-4-methoxyphenyl group, which would bind to the complementary AH-B system[6,7] of the sweet taste receptor by hydrogen bonding, whereas the aromatic ring A represents a lipophilic area responsible for the high intensity of the effect (Fig. 2).

A large number of analogues of these compounds have been synthesized and tasted. It was then recognized[8a] that the chelated *peri*-hydroxycarbonyl system is not necessary to give a sweet taste to the compounds, so that some recent work was

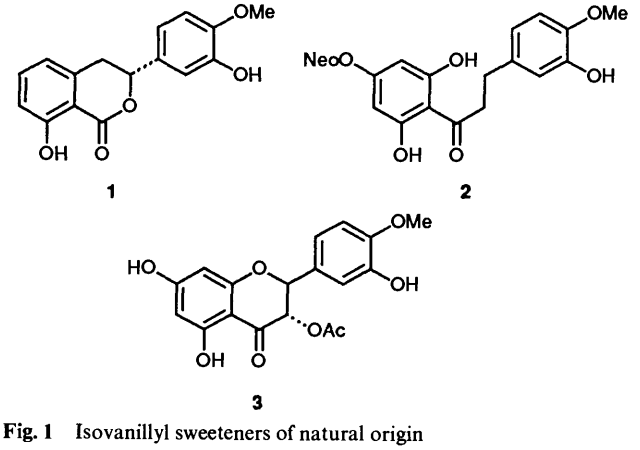


Fig. 1 Isovanillyl sweeteners of natural origin

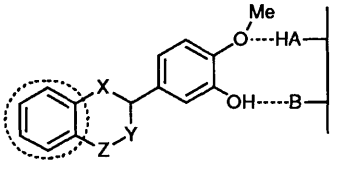


Fig. 2 The Shallenberger–Acree–Kier receptor model applied to isovanillyl sweeteners

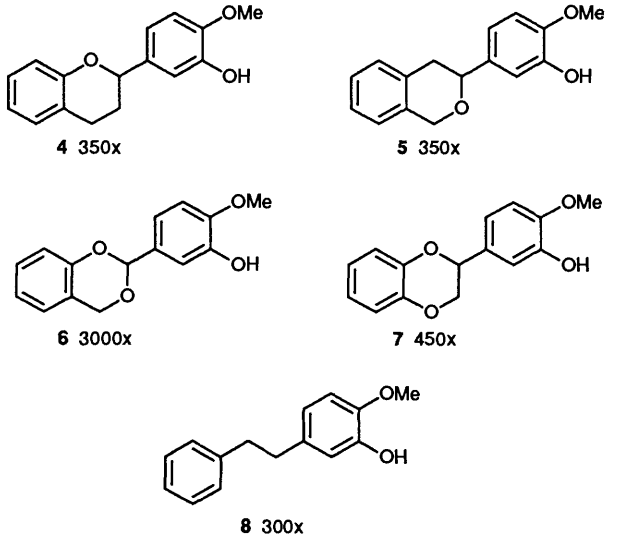


Fig. 3 Significant synthetic isovanillyl sweet compounds: **4** ref. 8(*a*); **5** ref. 9; **6** ref. 10; **7** ref. 11; **8** ref. 9

directed towards the synthesis of unsubstituted oxygen heterocycles: examples of these structures are reported in Fig. 3.

In general these compounds maintain the isovanillyl substituent; in fact, a systematic study in the case of dihydrochalcone derivatives has shown that only small changes are acceptable in this part of the molecule.[12] In contrast, important differences are acceptable in the heterocyclic B ring, and it has been shown that this ring is not indispensable for taste (see, for example, the bibenzyl derivative **8**).

As a first contribution in this field, we reported some time ago on the synthesis of the 1,4-benzodioxane **7**.[11]

We now report on the taste of some compounds in which the heterocyclic ring has been further modified. In particular we prepared the 1,4-benzodioxin **9** (a planar analogue of **7**), the

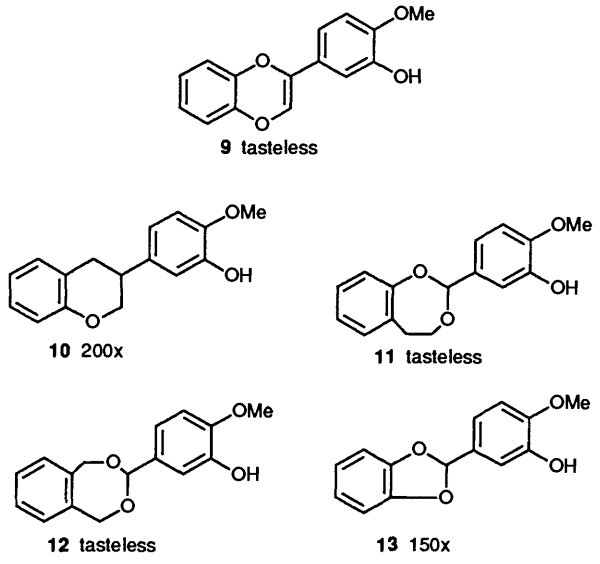
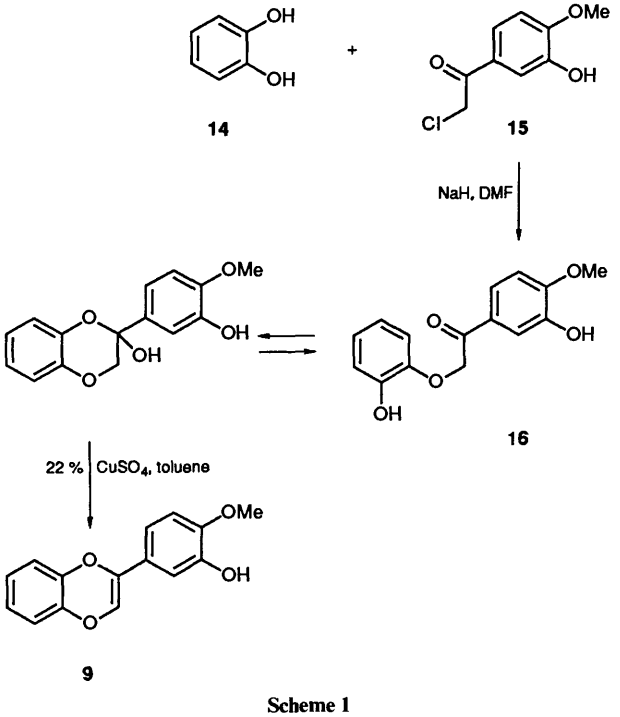


Fig. 4 Compounds synthesized and tasted in this work

isoflavan **10**, and some compounds in which the ring had been enlarged to seven members (**11** and **12**), or restricted to five members **13** (Fig. 4).

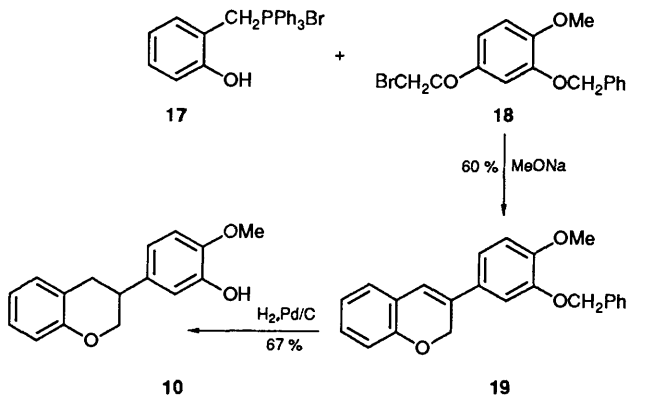
Results and Discussion

Synthesis of Compounds.—The 1,4-benzodioxin **9** was obtained in two steps (Scheme 1) by reacting 2-chloro-1-(3-hydroxy-4-methoxyphenyl)ethanone **15** with 1,2-dihydroxybenzene **14** in the presence of potassium carbonate in butan-2-one and by dehydrating the cyclic hemiacetal **16** with copper(II) sulphate in toluene.



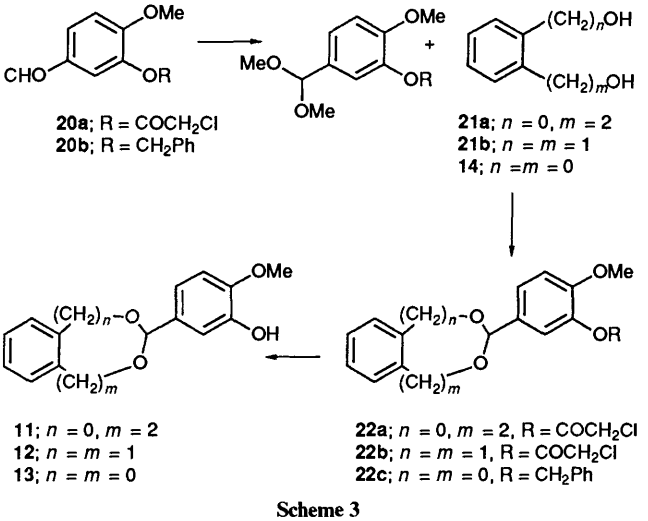
Scheme 1

The isoflavan **10** was obtained reacting 2-hydroxyphenylmethyltriphenylphosphonium bromide **17**[13] with 1-(3-benzyloxy-4-methoxyphenyl)-2-bromoethan-1-one **18**[14] in toluene in the presence of sodium methoxide (Scheme 2). The isoflavene obtained **19** was then reduced and deprotected by catalytic hydrogenation.



Scheme 2

For the synthesis of **11** and **12** (Scheme 3) isovanillin was protected as the chloroacetate and ketalized with trimethyl orthoformate and toluene-*p*-sulphonic acid in toluene. Without isolation the ketal was transketalized with the diol. The protecting group was then removed with $NaHCO_3$ in aqueous acetone. In the case of compound **13** these conditions produced some decomposition of the cyclic ketal, so that yields were improved using benzylisovanillin as a starting material.

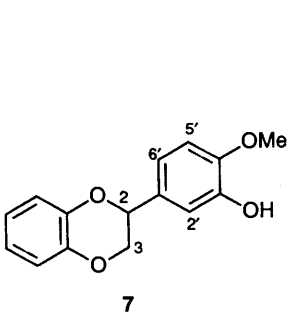


Scheme 3

Tasting of Compounds.—The solubility of the compound synthesized in water is low, but stable solutions can be obtained by predissolving them in ethanol and then diluting with deionized water. The new compounds were tasted for sweetness potency in comparison with a 3% sucrose solution. Relative taste potencies were determined from the ratios of the concentrations which gave the same sweet sensation. The results are shown in Fig. 4.

The 1,4-benzodioxin **9**, which contains a double bond in position 2,3 of the heterocyclic ring, is tasteless, thus confirming that also in the class of 1,4-benzodioxanes it is very important that ring B is not planar. Similar results had been observed with dihydroisocoumarins, flavones, coumarins and analogues of the bibenzyl **8**.[15]

The isoflavan **10**, which has the correct substitution on the aromatic ring and is not planar, is about 300 fold sweeter than sucrose. This compound completes the series of all the possible six-membered heterocyclic rings containing one or two oxygens in all the different positions. It can be seen that in all cases the compounds are sweet, that those containing one oxygen have about the same sweet taste potency, and that compounds containing two oxygens are sweeter, the 1,3 substitution being



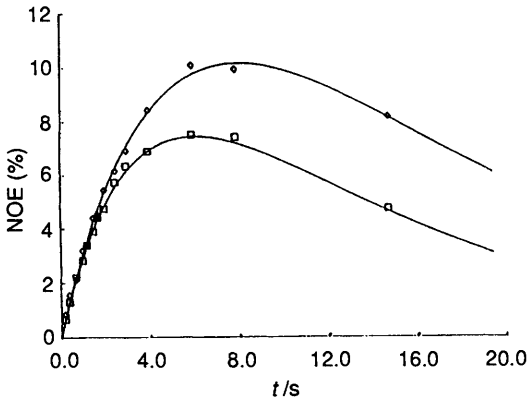


Fig. 5 NMR spectrum of compound **7** in [$^2H_6$]benzene. In the insert is shown the transient NOE of 2'-H (◇) and 6'-H (□) following inversion of 2-H.

the most effective. A comparative tasting of the isoflavan **10** and the 1,3-benzodioxane **6** showed that the former has less aftertaste at isosweet concentrations: the taste of **10** lingers a little, but the slight licorice aftertaste of **6** is lacking. The stability of **10** is also completely satisfactory, while compound **6** is unstable in water solution even at neutral pH.

The results of the tasting of compounds **11**–**13** show that the size of the heterocyclic ring is very critical: the two 1,3- and 2,4-benzodioxepines **11** and **12** are in fact completely tasteless, while in the case of a five-membered ring as in compound **13**, the sweetness is retained with a potency of 150.

Conformational Analysis.—Enough compounds have now been synthesized to make possible a study of the structure–activity relationship in this class. No correlation with the lipophilicity was found. It must be noted that the group contains isomeric compounds, which have the same (at least calculated) log *P*, but rather different sweetness potency. Electronic parameters for many heterocycles linked to the isovanillyl group are not available. Therefore, attention was devoted to the possible correlation with geometric parameters, already pointed out by other authors.[8a,16]

Any attempt to correlate structural parameters with sweetness would require an at least approximate knowledge of the conformation(s) of the active compounds. Very few (bibenzyl **8**[17] and neohesperidin dihydrochalcone **2**[18]) X-ray analyses have been performed on this class. On the other hand, useful information on the conformation in solution can be obtained from NMR spectroscopic studies. Therefore, a detailed study was performed on compound **7**.

The ^1H NMR spectrum of 1,4-benzodioxane **7** in [$^2H_6$]-benzene is shown in Fig. 5. The assignments were based on signal multiplicities and on the value of the measured coupling constants. The high value of $J_{2,3ax}$ (8.8 Hz) compared with $J_{2,3e}$ (2.5 Hz) indicates that the molecule exists in solution as a pure $^2H_3$ half-chair conformation (*i.e.* with the phenyl substituent in a quasi-equatorial orientation), in agreement with the molecular model obtained by energy minimization with molecular mechanics calculations (see later).

Table 1 Chemical shifts,[a] experimental[b] and calculated[c] ^{13}C longitudinal relaxation rates of 1,4-benzodioxane **7**

Atom	δ	$(1/T_1)_{exp}/s^{-1}$	$(1/T_1)_{calc}/s^{-1}$
C-2	74.89	0.368	0.368
C-3	69.40	0.713	0.766
C-5	(117.93)[d]	0.368	0.418
C-6	121.82	0.632[e]	0.719
C-7	121.82	0.632[e]	0.443
C-8	(117.51)[d]	0.368	0.417
C-2'	113.43	0.387	0.405
C-5'	110.67	0.400	0.402
C-6'	118.33	0.411	0.404

[a] Measured at 75.2 MHz and 25 °C in [$^2H_6$]benzene (24 mg cm^{-3}). Chemical shifts are from external $(CH_3)_4Si$. [b] The estimated accuracy is within 10%. [c] Calculated by using eqns. (5), (6) and (8) with the diffusion rate constants $D_x = 0.92 \times 10^{12}$ s^{-1}; $D_z = 3.0 \times 10^{12}$ s^{-1}; and $D_i = 6 \times 10^{12}$ s^{-1}. [d] Not assigned, values may be interchanged. [e] Due to peak overlapping, only the average of the relaxation rate is measured. The calculated average value is 0.581 s^{-1}.

In order to measure the preferred orientation of the phenyl ring with respect to the benzodioxane moiety, some transient nuclear Overhauser experiments, together with ^{13}C $1/T_1$ measurements, were performed. The NOE experiments consist of a semiselective inversion of selected protons and the measurement of the subsequent build-up of the NOE on neighbouring nuclei. A detailed discussion is reported in the appendix.

Table 1 shows the experimental ^{13}C $1/T_1$ relaxation rates,

Table 2 Experimental and calculated ^{1}H cross-relaxation rates for compound **7**

Protons	$(\sigma)_{exp}/s^{-1}$	$(\sigma)_{calc}$ [a]$/s^{-1}$
2, 3e	0.0180 ± 0.0013	0.0187
5′, 6′	0.0414 ± 0.0015	0.0467
2, 2′	0.0207 ± 0.0005	0.0184
2, 6′	0.0206 ± 0.0004	0.0184
3e, 2′	0.0045 ± 0.0005	0.0028
3a, 3e	0.15 ± 0.03	0.16
3a, 2′	0.0062 ± 0.0011	0.0042

[a] Calculated by means of eqns. (5), (6) and (7), with diffusion rate constants $D_x = 0.92 \times 10^{12}$ s^{-1}, $D_z = 3.0 \times 10^{12}$ s^{-1} and $D_i = 6.0 \times 10^{12}$ s^{-1}, with a dihedral angle $\varphi = -25°$.

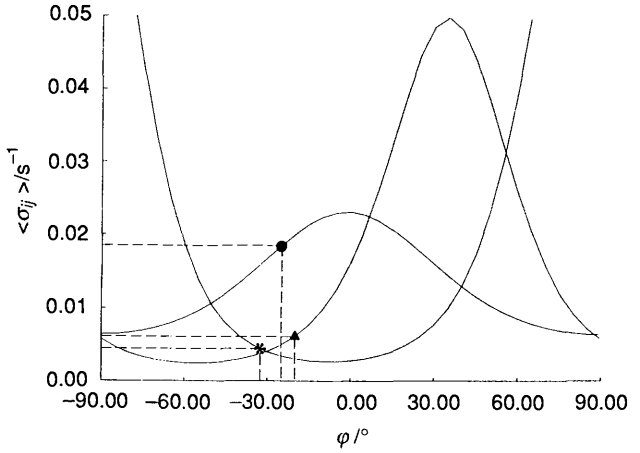


Fig. 6 ^{1}H cross relaxation rates $\langle\sigma_{ij}\rangle$ as function of the dihedral angle φ of compound **7** for 2-H,2′-H (●); 3a-H,2′-H (▲) and 3e-H,2′-H (✱)

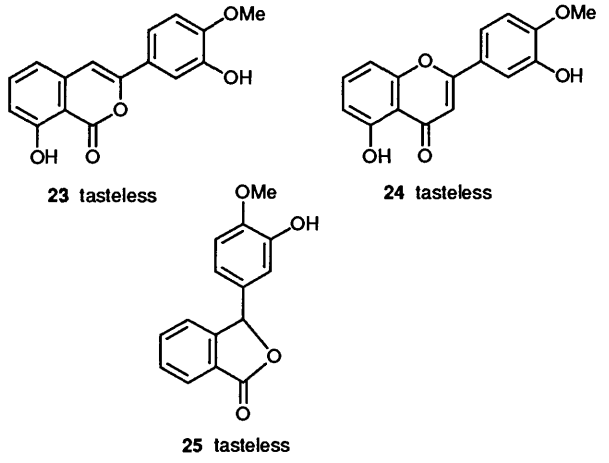


Fig. 7 Non sweet compounds: **23** ref. 9; **24** ref. 15; **25** ref. 20

together with the values, calculated from the derived molecular diffusion rate constants. Table 2 shows the experimental ^{1}H cross-relaxation rates σ_{ij}. The cross-relaxation rates, which are dependent on the orientation of the phenyl ring, *i.e.* $\sigma_{H2,H2'}$, $\sigma_{H2,H6'}$, $\sigma_{H3e,H2'}$ and $\sigma_{H3e,H6'}$, can also be calculated from the molecular diffusion rate constants, by assuming a model for the motion of the phenyl ring and by calculating an average value $\langle\sigma_{ij}\rangle$. We assumed the simplest model, consistent with our data and molecular mechanics calculations, *i.e.* a random jump of the phenyl ring between two equally populated orientations, whose dihedral angles differ by 180°. We have thus calculated $\langle\sigma_{ij}\rangle$ for different orientations of the phenyl ring by applying eqn. (7) of the appendix; in Fig. 6 $\langle\sigma_{ij}\rangle$ values are plotted as function of the dihedral angle φ (C2′–C1′–C2–H2). The preferred orientation of the phenyl ring obtained in this plot (by observing where the experimental values meet the corresponding lines) is $\varphi = -25°$. Table 2 compares the experimental σ_{ij} values with those calculated for $\varphi = -25°$.

The good agreement obtained in the case of this compound between experimental values and those calculated by molecular mechanics suggested that the geometries of other interesting compounds could be calculated in the latter way. Therefore we undertook molecular mechanics calculations on a series of sweet (**1–8**, **11**, **13**) and non sweet compounds (**9**, **11**, **12** and **23–25**, Fig. 7), using the MMPMI(85) program.[19]

Structures of compounds **6** and **7** were generated from standard bond lengths and angles and are fully optimized. The values obtained for the isovanillyl ring were used afterwards for the others, while standard bond lengths and angles were used for the benzocondensed rings. The X-ray structures were used as starting geometries for the calculations of **2** and **8**. In particular, in the solid state the Aryl–CO–CH_2–CH_2 chain of **2** is in a *gauche* arrangement, so that the overall shape of the aglycone is very similar to that of the tricyclic compounds.

In the case of six-membered ring B heterocycles, in the calculated most stable conformations, the isovanillyl substituent assumes an equatorial or quasi-equatorial orientation. This is consistent with the analysis of the ^{1}H NMR spectra of phyllodulcin **1** and of compounds **4** and **10**. Some time ago it was suggested by DuBois[16] (see, however, the discussion by Dick[8a]) that the 'bent' form of phyllodulcin **1**, with a quasi-axial orientation of the isovanillyl group, can be better accommodated in the Shallenberger–Acree–Kier receptor model. This is not true, however, for a more refined picture of the receptor, obtained by Temussi and co-workers[21] from calculations of the conformation of aspartame and other sweeteners.

In the calculated most stable conformation of all the tricyclic compounds the isovanillyl substituent appears nearly orthogonal to the plane of the benzo-condensed aromatic ring (angle β, Table 2). This study gave results which are fully consistent with those obtained by NMR spectroscopic studies on compound **7**. The possibility of a facile free rotation of the isovanillyl group around the bond linking it to the heterocyclic moiety was examined by calculating the energy of the conformations obtained by systematically rotating the group around the pivot bond. It appears that the energy barrier to the rotation is rather low ($\Delta E < 2$ kcal*), so that it is very likely that it can be overcome by the possible hydrogen bonding and/or polar interactions with the receptor. Therefore, we can suppose that in the active conformation interacting with the receptor the isovanillyl group can easily assume any orientation obtained by rotation around the pivot bond.

Incidentally, only the conformations with the isovanillyl ring almost parallel to the plane of benzocondensed ring fit well with the above-mentioned Temussi's model of the sweet-taste receptor, whereas conformations with the ring orthogonal or largely rotated should interfere with the so-called 'Shallenberger barrier'.[21a] If this hypothesis is correct, this barrier must have a sort of 'step', otherwise the lack of taste of the planar compounds could not be explained [Fig. 8(*a*)]. In the planar compounds, the aromatic ring is constrained in a position where Van der Waals interactions with the receptor would be too low [Fig. 8(*b*)].

As at present we still ignore the 'active conformation' in order to find a correlation between conformation and sweetness, we looked for geometrical parameters that could be independent from the rotation of the isovanillyl ring. These were found by superimposing, with a molecular modelling program, the

* 1 cal = 4.184 J.

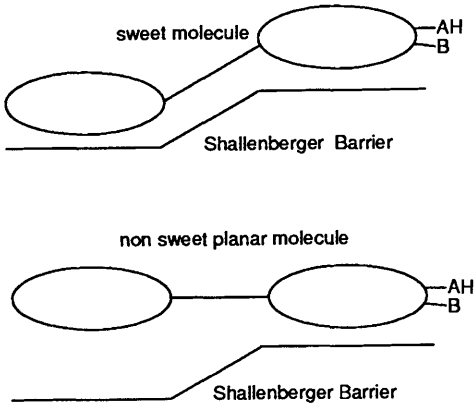


Fig. 8 Possible interaction of (*a*) sweet compounds and (*b*) non sweet compounds with the Shallenberger barrier

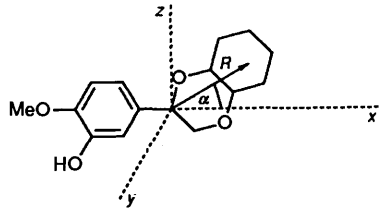


Fig. 9 Definition of the parameters α and *R* (left moiety superimposed for all compounds)

Table 3 Sweet taste potency, angle β, angle α and distance *R*

Compound	Angle β/°	Angle α/°	*R*/Å	Sweet taste potency
1	63	27.0	3.35	400
2	56	33.0	4.30	390
3	84	23.4	3.30	500
4	75	27.4	3.66	350
5	89	21.5	3.76	350
6	88	30.7	3.62	3000
7	80	27.1	3.61	450
8	11	36.4	3.06	300
9	58	19.0	3.60	0
10	90	21.0	3.73	200
11 (C)[a]	86	34.0	3.64	0
11 (TB)[b]	70	47.0	3.17	0
12 (C)[a]	86	27.0	4.24	0
12 (TB)[b]	78	48.0	4.28	0
13	87	39.0	3.26	150
23	70	15.8	3.71	0
24	85	20.9	3.66	0
25	83	64.7	2.85	0

[a] (C) = chair conformation. [b] (TB) = Twist boat conformation.

preferred conformations of all the compounds studied with the isovanillyl group occupying the same position in space, and assuming as the origin the nearest atom linked to the group. As the benzocondensed aromatic group is deemed to be the region of an important, and probably necessary, hydrophobic interaction with the receptor,[7] the distance *R* between the centre of this ring and the origin, and the angle α between the vector connecting this point with the origin and the axis of atoms 1–4 of the isovanillyl groups (Fig. 9) were calculated. The two parameters are independent on the rotation around the pivot bond.

Plotting sweetness potency against each of these two parameters (Table 3, Fig. 10) shows that the distance *R* alone cannot be directly correlated, whereas there is a range of values for angle α where the sweetest compounds are gathered. Plotting *R* against the angle α (Fig. 11) indicates a circular boundary which includes all the sweet compounds, with non-sweet ones scattered aside.

An exception is shown by the two 1,3- and 2,4-benzodioxepines (**11** and **12**). MM calculations indicated that the two conformations, a chair (C) and a twist-boat (TB), chosen as starting geometries for these two compounds, possess, once minimized, a very similar energy ($\Delta E < 1$ kcal). In the case of **13**, these results are consistent with a recent spectroscopic study of the 3-phenyl derivative.[22] Whereas the two TB conformations for both **11** and **12** fall outside the 'sweet region', this is not the case for the C conformations. Again, the difference in energy between the two conformers is well below the value of the possible interaction with the receptor.

This approach to correlate sweetness with molecular geometry for this class of compounds still has many drawbacks, and more data and compounds are necessary to attempt a quantitative correlation. Considering the number of compounds studied and their strict similarity, the results so far reached might have at least a heuristic value. Further work, including the synthesis of rigid compounds, is in progress to assess the scope of this analysis.

Experimental

M.p.s are uncorrected. ^{1}H NMR spectra were recorded on a Bruker WP-80 instrument, using tetramethylsilane as an internal standard, *J* values are given in Hz. Mass spectra were recorded on a Finnigan 4021 spectrometer. Anhydrous toluene was distilled on phosphorus pentoxide. Light petroleum is the fraction of b.p. 40–60 °C. Organic solvents were dried using Na_2SO_4.

2-(3-*Hydroxy*-4-*methoxyphenyl*)-1,4-*benzodioxin* (**9**).—Compound **16**[11] (280 mg, 1 mmol) was heated at reflux in anhydrous toluene (4 cm^3) in the presence of anhydrous $CuSO_4$ (0.25 g, 1.6 mmol) for 10 h. Water (10 cm^3) and ethyl acetate (10 cm^3) were added. The two layers were separated and the organic one was washed with water until colourless, then dried and concentrated under reduced pressure. The *title compound* **9** was purified by column chromatography (hexane–ethyl acetate 8:2) and washed with hot light petroleum (56 mg, 22%); m.p. 94 °C (Found: C, 70.2; H, 4.85. $C_{15}H_{12}O_4$ requires: C, 70.30; H, 4.72%); $\delta_H(CDCl_3)$ 3.83 (3 H, s, OCH_3), 5.58 (1 H, s, OH), 6.28 (1 H, s, 3-H) and 6.5–7.0 (7 H, arom.).

3-(3-*Hydroxy*-4-*methoxyphenyl*)-3,4-*dihydro*-2H-1-*benzopyran* (**10**).—Under nitrogen and with mechanical stirring, sodium methoxide (2.47 mol dm^{-3}; 2 cm^3) in methanol was dropped into a slurry of 2- hydroxyphenylmethyltriphenylphosphonium bromide (**17**)[13] (8 g, 17 mmol) in dry toluene (80 cm^3). After being stirred for 10 min, the mixture was added to α-bromoacetophenone (**18**) (6 g, 17 mmol). The yellow mixture was heated at reflux and sodium methoxide (2.47 mol dm^{-3}; 2 cm^3) was added dropwise during 30 min. The reaction mixture was refluxed for a further 5 h, then cooled. The salts were filtered, the solvents were evaporated and the residue was purified by column chromatography (hexane–ethyl acetate 9:1). The resulting white solid was washed with hot hexane to give 3-(3-benzyloxy-4-methoxyphenyl)-2*H*-1-benzopyran (**19**), (3.5 g, 60%); m.p. 102–103 °C; $\delta_H(CDCl_3)$ 3.87 (3 H, s, OCH_3), 5.02 (2 H, d, *J* 1, 2-H), 5.17 (2 H, s, OCH_2Ph), 6.62 (1 H, s, 4-H) and 6.8–7.5 (7 H, m, arom.). Compound **19** (1 g, 2.9 mmol) was hydrogenated in ethyl acetate in the presence of 10% Pd on carbon (100 mg). The mixture was filtered off, the solvent concentrated under reduced pressure and the crude compound chromatographed (hexane–ethyl acetate 7:3). The white solid was crystallized from cyclohexane to give 3-(3-*hydroxy*-4-

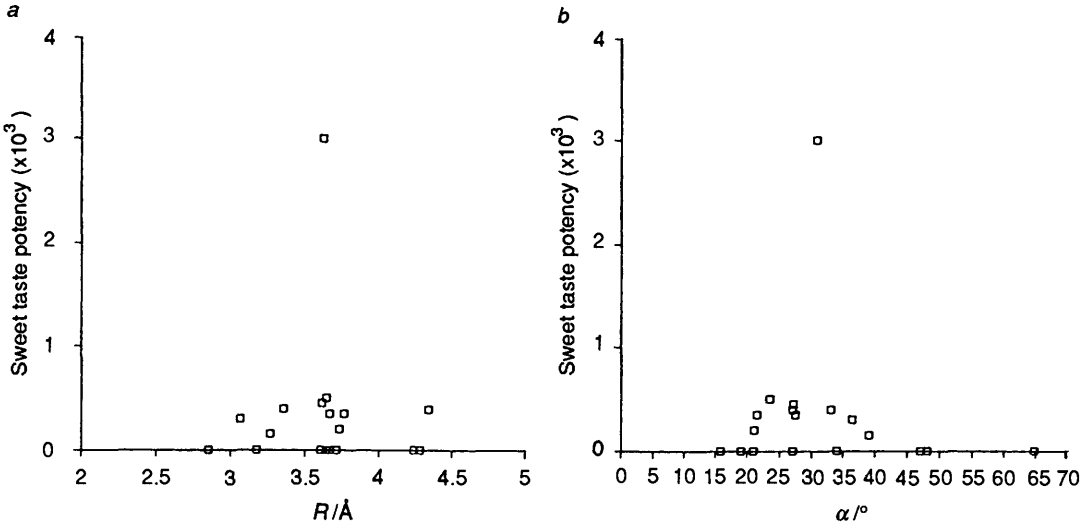


Fig. 10 Plots of sweet taste potency *versus* (*a*) angle α and (*b*) distance *R*

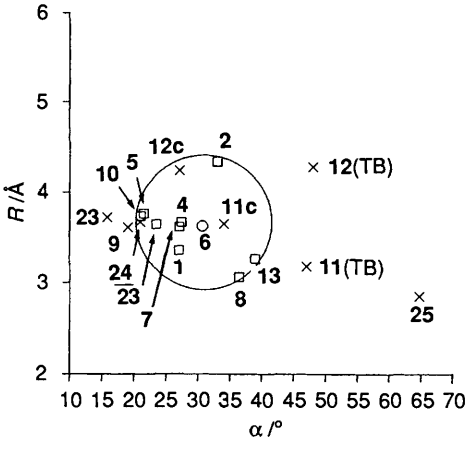


Fig. 11 Plot of angle α *versus R*. Type of compound: × non sweet, □ slightly sweet, ○ very sweet.

methoxyphenyl)-(2H)-3,4-*dihydro*-1-*benzopyran* (**10**) (500 mg, 67%); m.p. 95–96 °C (cyclohexane) (Found: C, 75.0; H, 6.3. $C_{16}H_{16}O_3$ requires: C, 74.98; H, 6.29%); $\delta_H(CDCl_3)$ 2.9–3.1 (3 H, 3-H and 4-H), 3.87 (3 H, s, OCH_3), 3.9–4.4 (2 H, m, 2-H), 5.6 (OH) and 6.7–7.25 (7 H, arom.).

2-(3-*Hydroxy*-4-*methoxyphenyl*)-4,5-*dihydro*-1,3-*benzodioxepine* (**11**).—A mixture of 3-chloroacetoxy-4-methoxybenzaldehyde (**20a**)[10] (2 mmol), trimethyl orthoformate (1.02 cm^3, 9.4 mmol) and toluene-*p*-sulphonic acid (0.11 g, 0.55 mmol) in toluene (80 cm^3) was heated at reflux for 4 h in a flask equipped with a Soxhlet apparatus filled with 4 Å molecular sieves. The formation of the dimethylacetal was followed by TLC. 2-(2-Hydroxyphenyl)ethanol (**21a**) (0.87 cm^3, 7.2 mmol) in toluene (3 cm^3) was added and the reaction mixture was refluxed for 12 h. After cooling, pyridine (0.25 cm^3) was added and the organic layer was washed with water (2 × 30 cm^3). The solvent was dried and concentrated under reduced pressure and the residue was purified by column chromatography (hexane–ethyl acetate 7:3). *Compound* **22a** is a white solid (1.16 g, 46%); m.p. 104 °C (from cyclohexane) (Found: C, 62.0; H, 4.9. $C_{18}H_{17}ClO_5$ requires: C, 61.98; H, 4.91%); ν_{max}/cm^{-1} 1795, 1240 and 1150; $\delta_H(CDCl_3)$ 2.77 (2 H, AB of ABXY, 5-H), 3.2–4.0 (2 H, XY of ABXY, 4-H), 3.90 (3 H, s, OCH_3), 4.36 (2 H, s, CH_2Cl), 5.50 (1 H, s, 2-H) and 6.9–7.7 (7 H, arom.). Compound **22a** (0.14 g, 0.4 mmol) was dissolved in acetone (1 cm^3) and treated with $NaHCO_3$ (0.12 g, 1.4 mmol) dissolved in the minimum amount of water. After 4 h at room temperature the mixture was diluted with water (5 cm^3) and extracted with dichloromethane (3 × 5 cm^3). The organic solution was dried and concentrated. The residue was purified by column chromatography (hexane–ethyl acetate 7:3). The white solid obtained was crystallized from cyclohexane (20 mg, 19%). 2-(3-*Hydroxy*-4-*methoxyphenyl*)-4,5-*dihydro*-1,3-*benzodioxepine* (**11**) had m.p. 119 °C (cyclohexane) (Found: C, 70.2; H, 5.9. $C_{16}H_{16}O_4$ requires: C, 70.57; H, 5.92%); $\delta_H(CDCl_3)$ 2.75 (2 H, AB of ABXY, 5-H), 3.2–4.0 (2 H, XY of ABXY, 4-H), 5.48 (1 H, s, 2-H), 5.60 (OH) and 6.8–7.4 (7 H, arom.).

3-(3-*Hydroxy*-4-*methoxyphenyl*)-1,5-*dihydro*-2,4-*benzodioxepine* (**12**).—The protected 2,4-benzodioxepine **22b** (1.46 g, 58%) was obtained in a similar way by reacting 3-chloroacetoxy-4-methoxybenzaldehyde (**20a**) (1.64 g, 7.2 mmol), trimethylorthoformate (1.02 cm^3, 9.4 mmol), toluene-*p*-sulphonic acid (0.11 g, 0.55 mmol) and 1,2-bis(hydroxymethylbenzene) (**21b**) (1 g, 7.2 mmol). Compound **22b**; m.p. 83–85 °C (from cyclohexane); $\delta_H(CDCl_3)$ 3.84 (3 H, s, OCH_3), 4.31 (2 H, s, CH_2Cl), 4.93 (4 H, s, OCH_2), 5.88 (1 H, s, 3-H) and 6.9–7.5 (7 H, arom.). Hydrolysis of this compound (200 mg, 5.7 mmol) gave 3-(3-*hydroxy*-4-*methoxyphenyl*)-1,5-*dihydro*-2,4-*benzodioxepine* (**12**) (0.12 g, 77%); m.p. 169–170 °C (from cyclohexane) (Found: C, 70.15; H, 6.0. $C_{16}H_{16}O_4$ requires: C, 70.57; H, 5.92%); $\delta_H(CDCl_3)$ 3.88 (3 H, s, OCH_3), 4.93 (4 H, s, OCH_2), 5.58 (OH), 5.88 (1 H, s, 3-H) and 6.8–7.2 (7 H, arom.).

2-(3-*Hydroxy*-4-*methoxyphenyl*)-1,3-*benzodioxole* (**13**).—In a similar way from benzylisovanillin (**20b**) (2 g, 8.2 mmol), trimethyl orthoformate (1.17 cm^3, 11.0 mmol), toluene-*p*-sulphonic acid (0.17 g, 0.9 mmol), and 1,2-dihydroxybenzene (**21c**) (0.91 g, 8.2 mmol), 2-(3-*benzyloxy*-4-*methoxyphenyl*)-1,3-*benzodioxole* (**22c**) was obtained (1.9 g, 70%), m.p. 117 °C (from cyclohexane) (Found: C, 75.25; H, 5.5. $C_{21}H_{18}O_4$ requires: C, 75.43; H, 5.43%); $\delta_H(CDCl_3)$ 3.90 (3 H, s, OCH_3), 5.12 (2 H, s, $PhCH_2O$) and 6.8–7.5 (13 H, arom.). This compound (0.5 g, 1.5 mmol) was hydrogenated in ethyl acetate in the presence of 10% Pd on carbon (50 mg). After filtration, the *title compound* **13** was purified by column chromatography (hexane–ethyl acetate 7:3) (0.30 g, 82%), m.p. 54 °C (from cyclohexane) (Found: C, 68.75; H, 4.9. $C_{14}H_{12}O_4$ requires: C, 68.84; H, 4.95%); $\delta_H(CDCl_3)$ 3.91 (3 H, s, OCH_3), 5.63 (OH) and 6.8–7.3 (8 H, arom.).

NMR Spectroscopic Study on 2-(3-*Hydroxy*-4-*methoxyphenyl*)-1,4-*benzodioxane* (**7**).—The sample used for ^1H NMR spectroscopic measurements was prepared by dissolving 2-(3-hydroxy-4-methoxyphenyl)-1,4-benzodioxane (**7**) (12 mg) in

[$^2H_6$]benzene (0.5 cm^3; Merck, >99% isotopic purity). The solution was thoroughly degassed by five freeze–thaw–pump cycles and the tube was sealed under vacuum. ^{1}H Transient NOE experiments were performed on a Bruker CXP300 spectrometer at 25 °C. The selective inversion was achieved by a low-power decoupler pulse (duration 25 ms). On- and off-resonance spectra were acquired in the inter-leave mode for each value of the mixing time. NOEs were calculated from the peak intensities measured in the difference spectrum. ^{13}C NME relaxation rate measurements were performed at 25 °C on a Varian XL-300 operating at 75.2 MHz on the same tube used for the NOE experiments. ^{13}C NMR spectroscopic chemical shift assignments were performed by means of a standard 2D shift correlation experiment. 64 FIDs of 1K each were acquired with a delay $1/2J_{C-H} = 0.0021$ s. They were subsequently Fourier transformed in absolute value after Lorentzian line-broadening of 5 Hz in f2, Gaussian-Lorentzian resolution enhancement in f1 and zero filling to 256 × 2K real data points.

Tasting of Compounds.—2-(3-Hydroxy-4-methoxyphenyl)-1,3-benzodioxane (**6**)[10] and 2-(3-hydroxy-4-methoxyphenyl)-1,4-benzodioxane (**7**)[11] were safe by toxicological evaluation. The compounds assayed in this work result from minor structural modification of them, and were not submitted to toxicological evaluation; however, they were tasted only once with the 'sip and spit' procedure at very low concentrations. The solutions for the tasting trials were obtained by dissolving the compounds (10 mg) in absolute ethanol (0.5 cm^3) and diluting to the desired concentrations with freshly distilled water. A panel of seven untrained people tasted the solutions in comparison with 3% sucrose in water containing the same amount of ethanol. If a compound was judged sweeter than the standard, it was subsequently diluted until an isosweet concentration was obtained. Panelists held the solutions in their mouth for some seconds, to reach the maximum intensity of taste.

Compounds **9**, **11** and **12** were tasteless at a concentration of 300 mg dm^{-3} and compounds **10** and **13** at a concentration of 150 and 200 mg dm^{-3}, respectively, were judged as sweet as the standard solution. As often happens in the case of isovanillyl sweeteners the taste is perceived after some seconds, but (especially in the case of **10**) no aftertastes were experienced.

Appendix

Analysis of Transient NOE Effect and ^{13}C T_1 *Experiments.*—The NOE is defined as the relative deviation in signal intensity, whenever some neighbour proton populations are perturbed by irradiation with a second RF field, *i.e.* selective inversion or saturation:

$$n = (I - I_0)/I_0 \quad (1)$$

where I and I_0 are the integrated intensities of the observed multiplet with and without the applied RF field, respectively.

In the case of transient NOE experiments, where the perturbation involves the inversion of a particular nucleus signal, the subsequent NOE build-up of the close in space nuclei follows a multi-exponential law, which derives from the integration of the corresponding Bloch equations (2):

$$\begin{aligned} dn_a/dt &= -r_a + \sigma_{ab} + \sigma_{ac} + \cdots \\ dn_b/dt &= \sigma_{ba} + r_b + \sigma_{bc} + \cdots \\ dn_c/dt &= \sigma_{ca} + \sigma_{cb} + r_c + \cdots \end{aligned} \quad (2)$$

where r_i are the longitudinal relaxation rates and σ_{ij} are the cross-relaxation rates. The integration is performed by diagonalization of the relaxation rate matrix Γ, whose elements are r_i and σ_{ij}. Thus, in matrix notation we have eqn. (3) where

$$\begin{aligned} [D] &= [T]^{-1}[\Gamma][T] \\ [n] &= [T]\,[\exp(-Dt)]\,[T]^{-1}\,[n]_{t=0} \end{aligned} \quad (3)$$

$[n]_{t=0}$ contains the initial condition of the experiment which is equal to the inversion factor for the irradiated proton and to zero for all the other nuclei.

The cross-relaxation rates σ_{ij} are dependent on the distance between the two interacting nuclei i and j, eqn. (4) where $\hbar$ is

$$\sigma_{ij} = 0.1\,(\mu_0/4\pi)^2\hbar^2\,\gamma_H^4\tau_c/r^6{}_{ij} \quad (4)$$

the reduced Planck constant, r_{ij} is the internuclear distance and τ_c is the molecular correlation time.

Eqn. (4) is valid only for time-independent distances, under non-extreme-narrowing conditions and isotropic molecular motions; when several conformations are present and they are rapidly interconverting in solution, the dipole–dipole interaction involves a time-dependent distance. The cross-relaxation rate $\langle\sigma_{ij}\rangle$ must therefore be computed directly from the spectral densities $J(w)$. The actual expression of $J(w)$ depends on the assumed motional model; in the case of isotropically reorienting molecules, the spectral densities are modulated by one single correlation time τ_c, as in eqn. (4), but in our case the non-spherical shape of the molecule, together with the presence of an internal movement, deserves the use of a more refined model.[2,3] Thus, we chose the case of anisotropic tumbling of the molecule, undergoing internal motions between two discrete conformations, *i.e.* a two-state jump model; in such cases the spectral densities are given by eqn. (5) where

$$J(w) = \sum_{a=-2}^{2}\sum_{n=0}^{1}\sum_{i=1}^{2}\sum_{j=1}^{2}\frac{f(n)}{w^2 + f(n)} \times \xi_i^0\xi_j^0\xi_i^n\xi_j^n \times \exp[ia(\alpha_i - \alpha_j)] \times \frac{d_{a0}(\beta_i)d_{a0}(\beta_j)}{r_i^3\, r_j^3} \quad (5)$$

$f_a(n) = [6D_x + a^2(D_z - D_x) + \lambda_n]$; D_x and D_z are the diffusion rate constants of the molecule around the principal axes of the diffusion tensor, assumed to be axially symmetric; d_{a0} (beta) are the reduced Wigner matrices; α and β are the Euler angles of the inter-nuclear vector with respect of the diffusion principal axes. The other coefficients describe the two-state jump model.

If the two conformations are equally populated, calling D_i the diffusion rate constant for the internal movement, we have eqn. (6). The spectral densities of eqn. (5) can be used to describe

$$\lambda = \begin{bmatrix} 0 \\ D_i \end{bmatrix} \quad \xi = \begin{bmatrix} 1/2 & 1/2 \\ 1/2 & -1/2 \end{bmatrix} \quad (6)$$

both the averaged proton-proton cross relaxation rates $\langle\sigma_{ij}\rangle$ [eqn. (7)] and the ^{13}C longitudinal relaxation rate T_1^{-1} [eqn. (8)] where the sum runs over all the directly attached protons.

$$\langle\sigma_{ij}\rangle = 0.1\,(\mu_0/4\pi)^2\gamma^4{}_H\hbar^2[J(2w) - J(0)] \quad (7)$$

$$(T_1)^{-1}{}_{DD} = 0.1\,(\mu_0/4\pi)^2\gamma^2{}_C\gamma^2{}_H\hbar^2\Sigma[J(w_C - w_H) + 3J(w_C) + 6J(w_C + w_H)] \quad (8)$$

Since the dominant dipolar relaxation for protonated ^{13}C atoms involves the directly attached protons, the interatomic distance is fixed and may be considered to be known. Therefore, it has been possible to derive, from the experimental ^{13}C

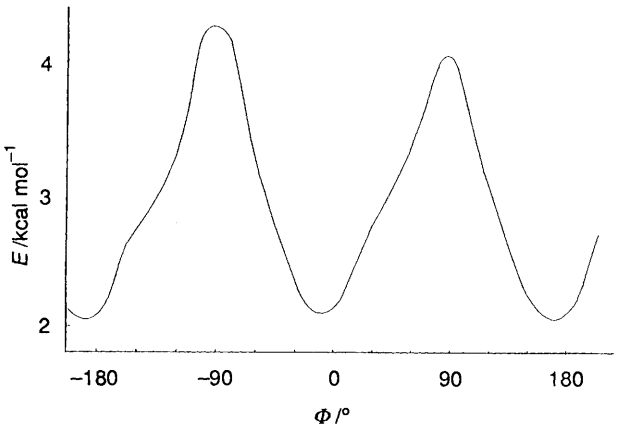


Fig. 12 Energies of compound **7**, calculated by molecular mechanics, as function of the dihedral angle φ

longitudinal relaxation rates, the values for the diffusion rate constants of eqn. (5), with the only assumption that the (unknown) diffusion principal axes are coincident with the principal axes of the inertia tensor, as calculated from the model geometry, derived by the MM calculations.

The diffusion rate constants D can, in turn, be used to calculate the $\langle\sigma_{ij}\rangle$ values as a function of different molecular geometries in order to find the best agreement between experimental and calculated values.

The experimental $\langle\sigma_{ij}\rangle$ values were derived by a non-linear least-squares fitting of the NOE data through the use of eqn. (3).

In the case of 1,4-benzodioxane (**7**), we measured the cross-relaxation rates between 2-H and 3e-H, 2′-H and 6′-H and between 6′-H and 5′-H. Fig. 5 shows the time development of the NOE on 2′-H and 6′-H signals following inversion of 2-H. The initial rate of build-up is equal for the two protons, although different maximum intensities are reached, suggesting that 2′-H and 6′-H interact with 2-H with the same cross-relaxation rate (see Table 1). We have also measured the cross-relaxation rates between 3e-H and 2′-H/6′-H. All these cross relaxation rates are dependent on the preferred orientation of the phenyl ring. The similarity of all the cross-relaxation rates involving 2′-H and 6′-H protons suggests that the phenyl ring either behaves like a free rotor or is freely jumping between two equivalent orientations. Actually, molecular mechanics calculations show evidence of a small rotational barrier of *ca.* 2 kcal mol^{-1} between two energetically equivalent minima, with rotational angles differing 180° from each other (Fig. 12). Thus, we may conclude that both MM calculations and NMR experiments are consistent with the two-state jump model and anisotropic molecular motions.

Given the distance dependence of the cross-relaxation rates, it has been possible, then, to derive molecular geometries which fit the experimental σ_{ij} by means of eqn. (7).

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