Sweet Taste Receptor Studies Using Model Interaction Energy Calculations

HANS-DIETER HÖLTJE*x and LEMONT B. KIER‡

Abstract □ Model interaction energy calculations using the monopoles-bond polarizabilities method are used to explain the different sweetness levels in a series of 2-amino-4-nitrobenzenes. The receptor model is isomorphic with the actual receptor feature.

Keyphrases □ Sweetness—model interaction energy calculations used to study sweet taste receptor □ Receptors, sweet taste—studied using model interaction energy calculations □ Monopoles-bond polarizabilities method—model interaction energy calculations of sweetness levels of 2-amino-4-nitrobenzenes □ 2-Amino-4-nitrobenzenes—determination of sweetness levels, model interaction energy calculations

A recent report (1) discussed the structural features of sweet-tasting molecules responsible for imparting the gustatory response. By beginning with the A–H/B theory (2) and recognizing the operation of stereospecificity in some sweet-tasting molecules (3), the location and nature of a third pharmacophore feature were sought. As a consequence of some conformational predictions using molecular orbital calculations, the pharmacophore was predicted to appear as shown in Scheme I (1).

The nature of this third structural feature, *X*, found in all major sweet-tasting molecules, was predicted to be a predominantly dispersion binding moiety. Support for this view came from the comparison of sweet taste potency, in a nitroaniline series, with a summed bond polarizability parameter approximating the ability of a particular moiety to engage in a dispersion bond. This comparison was made to describe in a more fundamental way the process involved, relative to a substituent parameter statistical comparison previously made with the same compounds (4). A very good correlation was found (r = 0.950 and r = 0.968) between the group polarizability and sweetness potency from two separate taste tests (5, 6).

The conclusion (1) that the third binding site of the sweet taste pharmacophore is engaging in a dispersion bond at a receptor site suggests that actual energies of interaction may be calculable using models of the receptor site.

BACKGROUND

In a recent study (7), a new approach to receptor mapping was initiated in which energies of interaction of parts of drug molecules were calculated, with model compounds simulating possible receptor features. This *receptor mapping using model interaction calculations* involves the assessment of the energy *versus* distance characteristics of various drug moieties in an interacting state, with possible receptor features. If the change of activity in a drug series can be ascribed primarily to the change in interaction energy (or affinity) at a particular drug moiety, then it may be possible to obtain a good correlation with activity if a receptor model simulating the actual physical situation is picked.

By using such an approach, the interaction energies and their changes due to molecular modification were calculated for the trimethylammonium and ammonium groups interacting with a series of small molecules, surrogate for amino acid side chains (7). The study led to the prediction that a reasonable choice of a receptor feature complimentary to the trimethylammonium group in acetylcholine is not a carboxylate or other polar moiety but is more likely an aromatic ring as found in phenylalanine, histidine, or tryptophan.

With the sweet-tasting nitroanilines, there is an opportunity to employ this same technique, receptor mapping using model interaction calculations, in a closely related series. In this study, the authors sought to identify receptor models giving interaction energies with the *X* pharmacophore feature in the nitroanilines correlating with the range of taste potency.

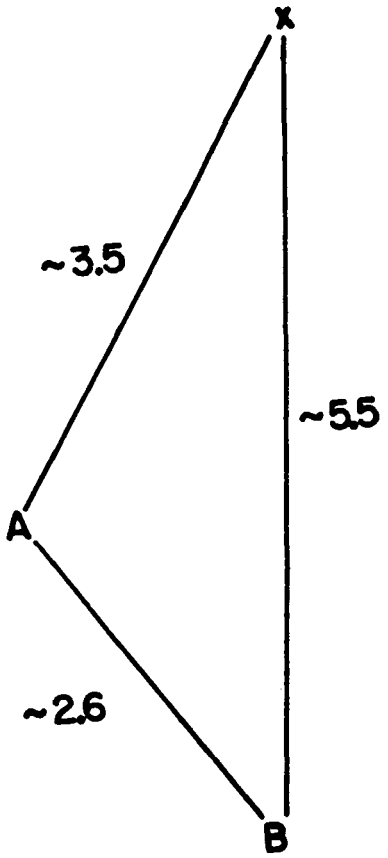
This nitroaniline series was examined previously using the extrathermodynamic approach to seek correlation with the sweetness level (4); a multicomponent equation was optimized when contributions representing an electronic influence and a hydrophobic bonding constant were included. It was found that the sweetness level depended upon the relative level of these contributions plus steric effects. In the present approach, the authors sought to visualize, quantitate, and correlate the enthalpy of interaction to determine if an alternative hypothesis to hydrophobic bonding can be supported.

The study model assumes that the A–H and B features of the sweet nitroanilines are anchored at the receptor with sufficient energy that the initial atom of the *X* moiety, the attached phenyl carbon atom, must be at a constant distance and relationship to the receptor feature complimentary to *X*. It is further assumed that preferred conformation of the *X* moiety is retained throughout the interaction. The sweet nitroanilines studied are shown in Table I. The general procedure is to select receptor models, derived from amino acid side chains, and to calculate the interaction energies between the *X* moiety and the receptor model for various modes of approach.

Whether some form of hydration of the receptor site or the model compound occurs should be considered. The nature and extent of solvation around molecular groups or atoms are not well understood. Arguments can be made for extensive or negligible intimate water interaction with various groups. In this case, it is felt that the point may be essentially moot for the following reasons. If only one water molecule intervened between the two interacting surfaces, then the van der Waals radii of the group and the water itself would prohibit an approach closer than about 8 Å, resulting in a negligible interaction energy between the groups *per se*. The energy of interaction involving the water and each interacting group, at 8 Å separation, would really be no more than the interaction energy of each isolated group and a complexed water molecule. Since a biological effect is measured, and since it is assumed that it is due largely to an interaction, it follows that the situation must involve the approach of two molecular groups, closer than 8 Å, in the absence of an intervening water molecule.

The favorable entropy change of water molecules surrounding the nonpolar portions of the sweet agent and the receptor may favor the association of these groups. The complexity of assessing this relative effect through this series is prohibitive. In essence, the model assumes a relatively constant influence from this sector while attempting to quantitate and correlate the enthalpic effect due to the interaction with the biological activity.

The finding of a good correlation between relative interaction energies at a common *X*–receptor model distance and sweetness level implies that the model is a good one, simulating what may be the actual receptor environment. Caution must be used in claiming that the receptor feature interacting with *X* has been identified. This may not be the actual case. However, the reported findings



Scheme I—Pattern of atoms imparting a sweet taste (glucophore)

identify a good candidate for that receptor feature, and this model may be useful in predicting and explaining the activity levels of new compounds.

This study considered only the amino acids as likely candidates for several reasons. The idea that a protein molecule is a good candidate for a receptor has wide currency. In the case of taste, some preliminary studies have, in fact, led to the isolation of a protein from the tongue which responds to sweet molecules in a parallel manner to their taste level (8). A firmer and, at the same time, a more general reason is that the amino acids in a protein are capable of significant perturbations by interacting with small ligands. Thus, the close approach of a ligand to a side chain can lead to a conformational perturbation in the side chain and in that residue backbone. This can easily lead to the massive disruption or alteration of the function of the entire protein, since its infrastructure is highly dependent upon influences across space in a folded molecule.

The steroid as a receptor model is nowhere near as good a choice. It is definitely rigid and unperturbable, except at positions joining it to other molecules. Furthermore, the steroid does not repeat itself in any kind of a mosaic, so the alteration of one steroid molecule will not result in an amplification or propagation of the effect through a macromolecule.

The phospholipid as a receptor candidate is a poorer choice for reasons similar to those described for the steroids. In addition, the phosphate group, being highly charged, presents a rather insensitive, nonselective feature to an approaching ligand. The high contribution of coulombic forces in the interaction with any ligand would not lead to a significant degree of discrimination between one ligand and another. Thus, the gradation in binding energies and the consequent gradation in biological effects observed would not be expected.

EXPERIMENTAL

For the calculation of the energies of interaction between the sweet compounds and the receptor models, the monopoles-bond polarizabilities method (9) was employed. The interaction energy is written in terms of the charge distributions of the two interacting molecules, approximated as point charges centered on the nucleus, and the polarizabilities of the bonds. These charges have been calculated by the CNDO–MO method (10). To this approach is added the repulsive energy component as the charge distributions begin to overlap. The interaction energy is the sum of the electrostatic (E_e), polarization (E_p), dispersion (E_d), and repulsion (E_r) energies.

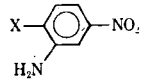
The electrostatic energy, E_e, is the work required or the energy gained when two charged points approach each other. The charge associated with each atom is assumed to be centered on the nucleus. The total electrostatic energy for two interacting molecules is then the sum of all pairs of atoms in the two molecules. Charges of like sign are repulsive while opposite signed atoms are attractive. This energy is related to the first power of the distance between two atoms, and it has a significant value even at long distances.

The polarization energy, E_p, is a measure of the distortion produced in the electron cloud of a bond by the presence of a charged atom in its vicinity. The ease with which a cloud of electrons can be perturbed is the polarizability. The magnitude depends on the relative freedom of the peripheral electrons from the influence of the atom nucleus. The distortion or polarization of the electrons in a bond by a nearby charged atom leads to an attraction that is related to the third power of the distance between the atom and the bond. This distance is a vector from the atom pointing to the midpoint of the bond. The energy of interaction of this type is a summation between all atom-bond pairs in the two molecules.

The dispersion energy, E_d, is the attractive force derived from the charge fluctuation in an orbital inducing a charge fluctuation in a nearby orbital. The two dipoles thus formed are complimentary and are attractive. The influence is related to the sixth power of the distance and is, therefore, highly dependent upon proximity and molecular similarity. The phenomenon is prominent in neutral nonpolar molecules. The ability of the peripheral electrons in the molecule to oscillate or be perturbed by induction is dependent upon their polarizability in the molecule. This molecular polarizability is approximated by the ionization potential of the molecule, *i.e.*, the energy required to remove an electron. The energy is derived from a summation over all atoms in both molecules.

All of these interactions would yield, upon calculation, an increasing energy of binding as atoms or molecules approached. Even coalescence of nuclei would calculate to be a favored process. But in reality, a repulsion occurs as the atoms approach. The repulsive energy quickly rises as atoms penetrate each other's electron domains or van der Waals radii. It is necessary then to correct the attractive energies with an expression simulating the repulsive energy, E_r. This equation is empirically derived, using the van der

Table I—Sweetness–Structure Relationships among 2-Amino-4-nitrobenzenes and Total Calculated Interaction Energies



X	Log Sweetness Relative to Sucrose	Calculated Total Binding Energy, at 4.25 Å Distance, kcal/mole (Scheme II)
OC_2H_7	3.61	5.40
$OCH_2CH{=}CH_2$	3.30	5.25
I	3.10	4.93
OCH_2CH_3	2.98	4.91
Br	2.90	3.79
$OCH(CH_3)_2$	2.78	4.91
Cl	2.60	3.59
OCH_3	2.34	4.22
CH_3	2.34	3.96
F	1.50	3.24
H	1.50	3.11

Table II—Contributions to Total Interaction Energy in Table I (kilocalories per mole)

R	E_e	E_p	E_d	E_r	E (total)
OC_3H_7	−0.129	−0.041	−6.545	+1.318	−5.397
$OCH_2CH{=}CH_2$	−0.122	−0.040	−6.136	+1.051	−5.254
I	+0.234	−0.032	−6.558	+1.425	−4.931
OCH_2CH_3	−0.115	−0.046	−5.921	+1.168	−4.914
Br	+0.225	−0.026	−5.056	+1.063	−3.794
$OCH(CH_3)_2$	−0.115	−0.046	−5.921	+1.168	−4.914
Cl	−0.200	−0.025	−4.609	+0.841	−3.593
OCH_3	−0.099	−0.051	−4.969	+0.902	−4.214
CH_3	−0.220	−0.045	−4.645	+0.948	−3.962
F	−0.191	−0.100	−3.607	+0.657	−3.241
H	−0.190	−0.036	−3.514	+0.632	−3.108

Waals radii in its formulation. These repulsions are summed over all interacting atoms in both molecules.

These calculations were used to predict interaction energies between DNA base pairs in the pioneering work of Rein and his collaborators (11–13). Hoyland and Kier (14) used it in the first application to the successful prediction of a molecular conformation, prostaglandin E_1.

The interaction energy between the two molecules can be written approximately as a sum of first- and second-order perturbation terms. The monopole-bond polarizabilities method of Claverie and Rein (9), as elaborated by Huron and Claverie (15), was utilized for the actual numerical calculations. Within this approximation, the interaction energy can be written in terms of the charge distributions of the two molecules approximated by point charges centered at the nuclei and the polarizabilities of the bonds, together with an algorithm for computing the repulsive energy component as the charge distribution begins to overlap. The actual working equations will be discussed.

For convenience, the two molecules are designated by subscripts 1 and 2. Let N_i and B_i be the number of atoms and bonds, respectively, in molecule i. Then the long-range interaction energy can be written as a sum of electrostatic *(E_e)*, polarization *(E_p)*, and dispersion *(E_d)* components. The explicit formulas are as follows:

$$E_e = \sum_{i=1}^{N_1}\sum_{j=1}^{N_2}\frac{q_i q_j}{R_{ij}} \qquad \text{(Eq. 1)}$$

$$E_p = -\frac{1}{2}\sum_{k=1}^{B_1}\boldsymbol{E}_k\bar{\mathbf{A}}_k\boldsymbol{E}_k - \frac{1}{2}\sum_{l=1}^{B_2}\boldsymbol{E}_l\bar{\mathbf{A}}_l\boldsymbol{E}_l \qquad \text{(Eq. 2)}$$

$$E_d = -\frac{1}{4}\times\frac{I_1 I_2}{I_1 + I_2}\sum_{k=1}^{B_1}\sum_{l=1}^{B_2}R_{kl}^{-6}\mathrm{Tr}(\bar{\bar{\mathbf{T}}}_{kl}\bar{\mathbf{A}}_k\bar{\bar{\mathbf{T}}}_{kl}\bar{\mathbf{A}}_l) \qquad \text{(Eq. 3)}$$

In Eq. 1, q_i is the charge at nucleus i and R_{ij} is the distance between the nuclei i and j. The quantity $\boldsymbol{E}_k$ in Eq. 2 is the electric field at the center of bond k (in molecule 1) due to the monopole charges q_j of molecule 2:

$$\boldsymbol{E}_k = \sum_{j=1}^{N_2} q_j \mathbf{R}_{jk} R_{jk}^{-3} \qquad \text{(Eq. 4)}$$

where R_{jk} is the distance from nucleus j to the midpoint of bond k, and $\mathbf{R}_{jk}$ is the vector of magnitude R_{jk} pointing from center j to the bond midpoint k. The quantity $\bar{\mathbf{A}}_k$ is the polarizability tensor for bond k.

The dispersion equation, Eq. 3, contains the average excitation energies, I_1 and I_2, approximated by the ionization potentials and a factor X which corrects for the fact that the usual London equation ($X = 1$) gives a result that is too small. The quantity R_{kl} is the distance between the midpoints of bonds k and l, and the tensor $\bar{\bar{\mathbf{T}}}_{kl}$ is defined as:

$$\bar{\bar{\mathbf{T}}}_{kl} = 3\frac{\mathbf{R}_{kl}\times\mathbf{R}_{kl}}{R_{kl}^2} - 1 \qquad \text{(Eq. 5)}$$

where 1 is the unit matrix. The symbol Tr in Eq. 3 indicates the trace of the quantity in parenthesis.

The final contribution that must be considered is the repulsion resulting from overlap of the charge distribution of the two molecules, designated as E_r. The Kitaygorodski (16) repulsion derived empirically from crystal energies of hydrocarbons, but slightly modified by Huron and Claverie (15), was used. This relationship is:

$$E_r = 30{,}000\sum_{i=1}^{N_1}\sum_{j=1}^{N_2}\exp[-5.5R_{ij}(V_iV_j)^{-1/2}] \qquad \text{(Eq. 6)}$$

where V_i is the van der Waals radius of atom i.

Standard bond lengths and angles are assumed. The ionization potentials are derived from the CNDO/2 calculations. The polarizabilities are taken from Denbigh (17) or leFevre (18). The van der Waals radii are as follows: H = 1.2, C = 1.6, C (aromatic) = 1.8, N = 1.5, O = 1.4, F = 1.4, Cl = 1.8, Br = 2.0, and I = 2.2.

Several preliminary calculations were made using the derivatives listed in Table I and amino acid side-chain models. A correlation was sought between the interaction energies at a constant X–receptor distance through the series and the sweetness level. Only the model compound surrogate for the tryptophan side chain gave a significant correlation. Accordingly, this receptor model was examined further by varying the mode of approach of X to the 3-methylindole surface. The mode of interaction and the X–receptor distance was optimized to give the best correlation to total interaction energy and sweetness.

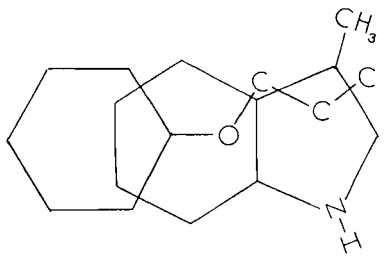
A second criterion was used to optimize the X–receptor interaction model. If it is assumed that the sweetness level in this series is due entirely to the affinity of X for the receptor, then the potency ratio of the extremes in the series can be related to the difference in binding energies of the extreme cases with the equation:

$$\Delta E = -RT\ \ln\ \rho \qquad \text{(Eq. 7)}$$

The term ρ is the potency ratio, 127 in the present case. This expression has been used (19, 20) to relate affinities to potencies. From the relative potencies in Table I, the difference in binding energies necessary to account for the extreme potency difference was calculated to be 3.0 kcal/mole. These interaction calculations should agree as closely as possible with this range of binding energies for the two extreme cases of sweetness potency to ensure that the model is reasonable.

RESULTS

The calculations show that 3-methylindole, simulating the side chain of tryptophan, is the best receptor model for the X moiety of sweet nitroanilines among several amino acid side-chain models. The optimum relationship between X and this model is shown in Scheme II. Table I lists the total calculated interaction energies for each compound at the optimum X–receptor distance of 4.25 Å;



Scheme II—Optimum mode of interaction of the X moiety of the sweet nitroanilines and 3-methylindole, simulating tryptophan as a receptor site. The X in this case is propoxy.

Table II lists the contributions of the electrostatic, polarization, dispersion, and repulsion energies to the total energy (Table I). The mentioned distance separates the phenyl ring carbon attached to X and the plane of the indole ring.

Relating the energies to the sweetness level (Table I) gives a correlation coefficient of $r = 0.887$. The interaction energy difference predicted between the two extreme cases is 2.3 kcal/mole, compared to the 3.0 kcal/mole predicted from the expression previously described.

DISCUSSION

The calculations reveal a fairly good correlation relating a biological activity with a single, relatively fundamental, molecular property. The property is a dynamic one, namely the total interaction energy with a model compound simulating the receptor feature. Accordingly, it can be concluded that the choice of receptor is fairly good under the circumstances.

The interaction energy difference calculated for the two extreme sweetness cases, 2.3 kcal/mole, is of the same magnitude as the energy difference predicted from the thermodynamic expression, 3.0 kcal/mole. The latter value presumes that all biological activity variation is due to differences in the energies of binding.

An interesting observation can be made relating to the value of the correlation and the extremes of binding energy predicted in the series. The correlation coefficient, $r = 0.887$, indicates that 79% of the variation has been accounted for in the relationship. By comparing the calculated interaction energy spread of 2.3 kcal/mole with the energy spread predicted from the thermodynamic expression of 3.0 kcal/mole, it can be said that the results imply that 77% of the biological activity ratio of the extreme cases is a result of interaction energies considered in the model.

The similarity of these values, derived from independent aspects of the study, indicates that the model is correctly simulating the part of the drug–receptor binding that can be ascribed to the calculated interaction energies. From Table II it is clear that the overwhelming contribution to those energies is, as predicted, due to dispersion forces. Electrostatic forces are relatively small.

The potential value of these results and of the general method in other cases lies in the possibility that a model can now be constructed that is isomorphic with the actual drug–receptor system. Such a model may be useful in explaining activity and designing new drugs.

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GLC Determination of Plasma Levels of Warfarin

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Abstract □ A novel method for the quantitative estimation of warfarin in plasma is described. Plasma containing warfarin to which a known amount of phenylbutazone is added as internal standard is acidified and extracted with ethylene dichloride. The drug and the internal standard are then back-extracted into alkali which, in turn, is acidified and reextracted with ethylene dichloride. The organic extract, after washing with phosphate buffer (pH 7.2), is evaporated and the evaporated extract is reacted with an ethereal solution of diazomethane (100 μl). The reacted mixture is evaporated and then dissolved in 25 μl of carbon disulfide. Aliquots (2–3 μl) are injected into a gas chromatograph equipped with a flame-ionization detector. The methyl derivatives of warfarin and the internal standard give sharp, well-separated, symmetrical peaks. The method is of sufficient sensitivity to determine plasma levels in humans after single doses (20 mg) of warfarin (sensitivity of 0.25 μg/ml).

Keyphrases □ Warfarin—GLC determination in plasma □ GLC—analysis, warfarin in plasma

Analysis of warfarin from biological fluids by spectrophotometric (1), fluorometric (2, 3), and TLC (4) methods have been described. The O'Reilly *et al.* (1) method, used by several investigators (3, 5–8), was modified by Welling *et al.* (9) to make it more sensitive. This modified method has been successfully employed to study the *in vivo* and *in vitro* availability of commercial warfarin tablets (10).