A Molecular Theory of Sweet Taste

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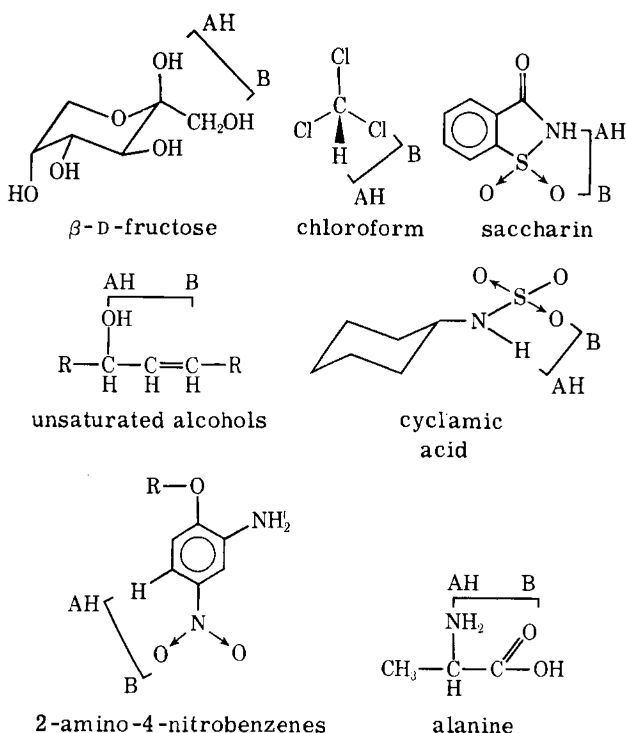
Abstract □ A study of several stereoselectively sweet amino acid molecules has led to a prediction of the presence and approximate location of a third structural feature, relative to the currently postulated A—H/B features in the glucophore. An examination of several unrelated sweet molecules supports this glucophore hypothesis. A relationship between a polarizability parameter and sweetness level in a series of substituted nitroanilines leads to the hypothesis that this third binding site may be involved in dispersion bonding with an appropriate receptor feature.

Keyphrases □ Sweetness of molecules—molecular theory, three structural features □ Molecular theory of sweetness—structural features, relationship between polarizability parameter and sweetness level □ Amino acids—prediction of sweetness, molecular features □ Structure of molecules—prediction of sweetness □ Glucophores—prediction of sweetness, three structural features

A description of the molecular features imparting a sweet taste to a molecule has been sought for many years (1–4). A large number of molecules have been tested for this property in an effort to deduce structure–activity relationships. With the advent of low calorie foods, artificial sweetening agents have taken on significant commercial interest, leading to the marketing of large quantities of potent compounds. The discovery that the leading sweetening agents cyclamic acid and saccharin may induce carcinoma has jeopardized their place in commerce, creating a high demand for a safe sweetening agent.



Scheme I—Various sweet molecules showing the A—H and B structural features involved cited by Shallenberger and Acree (5)

DISCUSSION

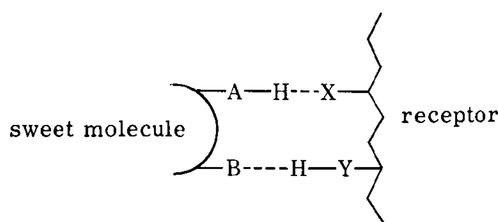
A theory on structural features responsible for sweet taste was proposed by Shallenberger and Acree in 1967 (5). They defined a basic structural unit common to sweet molecules. The unit was concluded to be an electronegative atom B and a polarized system A—H. The A–B distance was found to be 2.5–4.0 Å in sweet molecules. In sweet sugar glycols, for example, it was found that a vicinal pair of hydroxyl units was *gauche* while in nonsweet sugars, eclipsed or *trans*-glycol pairings were present. This observation is consistent with the A–H distance of 2.5–4.0 Å. An examination of a wide variety of sweet molecules illustrates the theory (Scheme I), and led Shallenberger and Acree (5) to postulate a general theory of receptor interaction involving features A—H and B in the sweet molecule (Scheme II).

It appears evident from a number of independent studies that a third binding site in a molecule is necessary for a potent sweet taste response. Thus, Deutsch and Hansch (6) concluded that a hydrophobic bonding area is necessary in a series of potent sweet compounds based on a substituent effect correlation. The observation that some D-isomers of amino acids are sweet while the L-isomers are not indicates a stereoselective receptor and, hence, at least three potential binding sites on the molecule (7). The identification of this third binding site and some information on its nature would provide a rationale for the design of new potentially useful sweet agents (8).

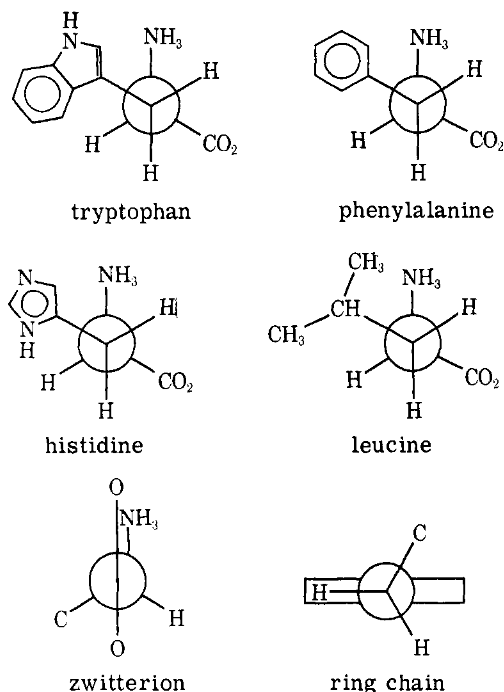
The definition of pharmacophores for various pharmacological activities has been of interest for some time. The approach has been to predict the molecular conformation of chemically different but pharmacologically equivalent molecules using theoretical chemical methods (9). When consideration of the molecules in their preferred conformations revealed a common pattern of similar charged atoms or groups, it was postulated that this pattern is relevant to receptor features and, accordingly, this pattern as a pharmacophore was proposed. In this way, Kier and his coworkers predicted the pharmacophores for muscarinic, nicotinic, histamine-H_1 and histamine-H_2, serotonin, α-adrenergic (9), β-adrenergic (10), and γ-aminobutyric acid (11), among others.

The studies on sweet molecules began with the molecular orbital prediction of the conformation of D-tryptophan, reported by Solms (7) to be 35 times the sweetness of sucrose. The molecule is of particular interest since the L-form is without any sweet taste; hence, a stereoselectivity is exhibited. It was the intent to consider this and other stereoselective sweet molecules in an attempt to find a common third binding feature. Extended Hückel theory calculations (12) were employed using parameters and dimensions previously used in amino acid residue conformation studies (13–15). The results are illustrated in Scheme III. The D-amino acids histidine and phenylalanine, also reported to be stereoselectively sweet but of a lower degree than D-tryptophan (Table I), were then considered.

An examination of these three amino acid zwitterions reveals that theoretical calculations predict a common conformational preference for the NH_3—CH—COO moiety. Furthermore, the N to O atom distance is predicted to be 2.6 Å, agreeing with the A–B di-



Scheme II—Postulated interaction of molecular sweet feature and receptor, according to Shallenberger and Acree (5)



Scheme III—Calculated preferred conformations of tryptophan, phenylalanine, histidine, and leucine zwitterions.

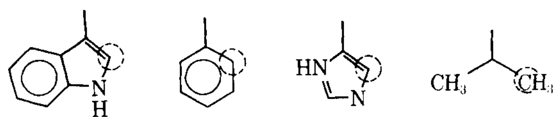
mension postulated by Shallenberger and Acree (5) to be a necessary condition for sweetness. If one relates an N—H group to the A—H feature and an O atom to the B feature in the hypothesis of Shallenberger and Acree, one then can offer an explanation of the sweet characteristics of these three amino acids.

A search for a common third binding site in these three molecules reveals a ring position in each side chain to be superimposable when the NH_3—CH—COO moiety is held in a common position. These common positions are illustrated in Scheme IV. Chemically, these three ring positions are electron rich. The 2-position in indole and related compounds was implicated as a localized π-π charge-transfer donor (16), a concept more recently supported by molecular orbital calculations (17) and NMR evidence (18). The *ortho*-position in the phenyl ring was also shown to have a high potential for localized charge transfer or susceptibility to electrophilic attack (19, 20). Finally, the position of the imidazole ring, designated in Scheme IV, was shown to be the most electron rich of the ring carbons (21).

It appeared evident from this preliminary consideration that a common third site in these three sweet molecules involved an electron-rich position capable of undergoing electrophilic attack, engaging in localized charge transfer, or capable of participating in some type of bonding involving the electron component such as a dispersion interaction.

This latter idea gained some support when the calculation of the preferred conformation of the moderately sweet D-leucine (Scheme IV) revealed that a methyl group of the side chain prefers a relationship to the NH_3—CH—COO moiety identical to that just described. This finding suggests that the third binding site of sweet molecules may be involved in a dispersion bonding at the receptor. The methyl group would be a weak participant in this type of bonding; hence, its weaker activity than the other D-amino acids might thus be explained.

At this point, a provisional hypothesis of molecular features imparting a sweet taste may be constructed. A necessary structural



Scheme IV—D-Amino acid side-chain features found to occupy a common spectial area when the NH_3—CH—COO moiety of each (Scheme III) is held in a common position

Table I—Amino Acid Sweetness Relative to Sucrose[a]

Amino Acid	Taste (Times Sucrose) L-Isomer	D-Isomer
Tryptophan	Bitter	Sweet (35×)
Phenylalanine	Bitter	Sweet (7×)
Histidine	Bitter	Sweet (7×)
Tyrosine	Bitter	Sweet (6×)
Leucine	Bitter	Sweet (4×)
Alanine	Sweet (1.5×)	Bitter
Glycine	Sweet (1.5×)	Sweet (1.5×)
Arginine	Flat	Slightly sweet
Aspartic acid	Flat	Flat
Isoleucine	Flat	Flat
Lysine	Flat	Flat
Proline	Flat	Flat
Serine	Flat	Flat
Threonine	Flat	Flat
Valine	Flat	Flat
Cysteine	Sulfurous	Sulfurous
Glutamic acid	Unique	—
Methionine	Sulfurous	Sulfurous

[a] *Reference 7.*

feature certainly appears to be the A—H/B system proposed by Shallenberger and Acree (5). In our view, the A—H feature is better defined as a polarized bond rather than a particularly labile or acidic hydrogen. This is supported by the calculations of the amino acids shown in Scheme IV. In addition, the calculations point to a common third structural feature capable of charge transfer or dispersion bonding. Its position relative to the A—H/B system is shown in Scheme V.

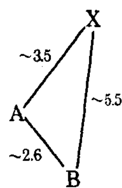
A preliminary test of this proposed structural pattern or glucophore can be made by considering the amino acid isomers in Table I. One finds a low level of sweetness comparable to glucose in the case of L-alanine, glycine, and D-arginine. It is conceivable that the A—H/B system in these molecules is sufficient to impart a basal level of sweetness of the same order of magnitude as sucrose. The other amino acid isomers are all nonsweet. This may be explained in two ways. In the case of D- or L-lysine, L-arginine, cysteine, methionine, serine, or the acids, the presence of a nonhydrocarbon functional group in the side chain deters the molecule from proper orientation at all three receptor sites postulated in Scheme V. The remaining nonsweet amino acids in Table I may be so because they do not have a side-chain atom or group remote enough from the amino acid backbone to occupy the proposed third receptor site.

This provisional test of the hypothesis is encouraging; however, it would be premature to accept it without a detailed examination of a larger group of known sweet compounds. Accordingly, the author searched for this third binding site in the currently identified prominently sweet molecules (Scheme VI). The five molecules depicted here constitute the most potent sweet molecules, ranging from 500 to 4000 times the sweetness of sucrose. They are also compounds for which a reasonable estimate of the preferred conformation can

Table II—Log Relative Sweetness *versus* Hansch's π, and Group Polarizabilities

R	$\Sigma\alpha$[a]	π[b]	Log *RS*[c]	Log *RS*[d]
$OCH_2CH_2CH_3$	6.653	0.98	3.613	3.699
$OCH_2CH{=}CH_2$	6.458	—	3.301	—
I	5.57	1.20	3.097	3.097
OCH_2CH_3	4.868	0.48	2.978	3.146
$O(CH_2)_3CH_3$[e]	(8.438)	—	3.000	—
Br	3.64	0.86	2.903	2.903
$OCH(CH_3)_2$[e]	(6.653)	—	2.778	—
Cl	2.53	0.71	2.602	2.602
OCH_3	3.08	−0.02	2.342	2.519
CH_3	2.44	0.56	—	2.519
F	0.705	0.14	1.502	1.602
H	0.655	—	—	1.602

[a] Values from J. L. Webb, "Enzyme and Metabolic Inhibitors," vol. 1, Academic, New York, N. Y., 1963, p. 220. [b] *Reference 6.* [c] Verkade, Van Kijk, and Meerburg, *Rec. Trav. Chim. Pays-Bas*, **50,** 346 (1946). [d] J. J. Blanksma and D. Hoegen, *Rec. Trav. Chim.*, **65,** 333(1946). [e] These data are not included in regression analysis discussed in text.

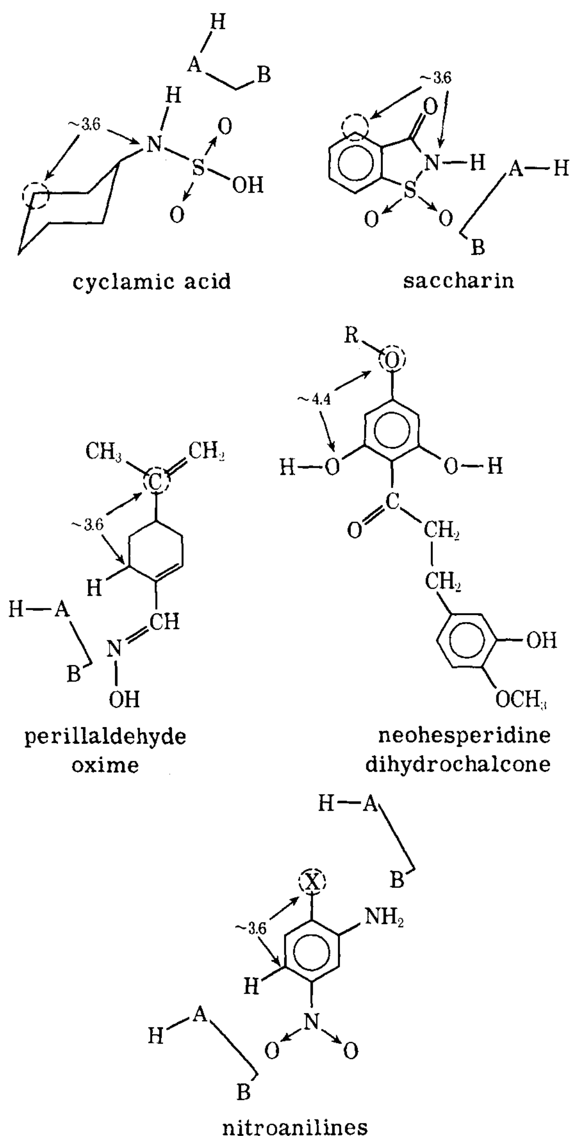


Scheme V—Pattern of atoms imparting a sweet taste (glucophore), postulated from this study

be made. Missing from this list is the dipeptide aspartylphenylalanine methyl ester (22). At this time, a reasonable conformation for the complete dipeptide molecule cannot be proposed.

The molecules in Scheme VI reveal a recurring pattern of A—H/B and a common third binding site. This pattern conforms very well to the hypothesized glucophore in Scheme V. The diverse nature of the hypothesized third binding site appears to implicate a dispersion bonding interaction of this position or this region with a receptor feature.

Further support for the idea of a dispersion bonding at the postulated third site in the glucophore comes from Deutsch and Hansch's (6) consideration of the substituted nitroanilines (Table II). They found a codependence of sweetness on the Hammett constant and the hydrophobicity constant. In this case, one may reinterpret the hydrophobicity constant as paralleling the polarizability of the group or, more generally, as roughly paralleling the potential ability for a dispersion interaction.



Scheme VI—Identity of third structural feature comprising the postulated glucophore (Scheme V) in several sweet molecules

A superior relationship can be found between relative sweetness in the nitroaniline series and a summation of bond polarizabilities (Table II). The correlation coefficient relating $\Sigma\alpha$ with Blanksma's sweetness data is $r = 0.968$, with the standard deviation of $S = 0.1853$. The situation is similar when comparison is made with Verkade's data where $r = 0.950$ and $S = 0.2205$. This is the first example of a biological effect correlating in a significant way with a single parameter reflecting dispersion bonding.

The group polarizability value here reflects the potential of the group to engage in a dispersion bond but, of course, does not consider optimum distances or the structure of the interacting receptor feature. The apparent importance of the shape of the structural feature in this position is illustrated in Table II in the case of the $O(CH_2)_3CH_3$ and $OCH(CH_3)_2$ derivatives. These moieties have a high polarizability but their extended or branched character must deny the molecule optimum interaction with the receptor; hence, the two derivatives have a depressed level of sweetness.

The relationship just described for the nitroanilines supports the hypothesis of the glucophore (Scheme V) and the idea that the third binding site is a region in the molecule capable of undergoing dispersion bonding at a receptor. The dimensions shown in Scheme V then are to be interpreted as distances from the A and B features to the initial atom of a chain of atoms probably involved in this bond.

The utility of this hypothesis is obvious. The receptor equivalents for the A and B features are illustrated in Scheme VI. The receptor equivalents, approximated physical characteristics, and ranking of receptor-active third site structural features are evident from Table II. Hopefully, the design of new potent sweet molecules can be stimulated by this hypothesis.

EXPERIMENTAL

The calculations of the conformations of tryptophan, phenylalanine, histidine, and leucine were carried out using the extended Hückel theory of Hoffmann (12), using parameters previously employed (9) and bond lengths and angles employed in amino acid studies (13–15).

An alternate all-valence electron molecular orbital method is available. This method, known as the "complete neglect of differential overlap" method, employs self-consistent field formalism (23). In spite of an initially superior theoretical framework, the complete neglect of differential overlap method neglects differential overlap and, hence, underestimates repulsive interactions. The extended Hückel theory method exaggerates these. As a consequence, the complete neglect of differential overlap method often leads to the prediction of equal energies for *trans-* and *gauche-*conformations, while the extended Hückel theory method predicts a sharply defined energy minimum with exaggerated barriers. The complete neglect of differential overlap method also exaggerates the contribution of hydrogen bonding whenever this can occur in the molecule. The complete neglect of differential overlap method, however, is superior for charge distributions.

In a careful analysis of the two methods, Hoyland (24) concluded that for the prediction of conformation only, the extended Hückel theory method was probably superior. In our hands, this method has consistently produced results in agreement with available or subsequently available experimental aqueous solution data (25). The record of complete neglect differential overlap calculations on these same molecules has not been as good. As a consequence, we continue to use the extended Hückel theory method with the belief that it is the best available semiempirical molecular orbital method to predict conformation of large molecules.

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Sensitive Spectrophotometric Method for Determining Methadone in Biological Specimens

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Abstract □ The extensive use of methadone for the treatment of opiate addiction increases significantly the need for quantitative determination of methadone in urine and tissues. Existing spectrophotometric methods are limited in sensitivity by the low molar absorptivity of this compound (ϵ = 554 in 0.1 *N* HCl, λ = 292 nm.). Results demonstrate that a markedly enhanced sensitivity may be achieved by oxidizing the methadone to benzophenone (ϵ = 18,713 in *n*-heptane, λ = 247 nm.). Methadone is extracted into *n*-hexane at an alkaline pH and then back-extracted into dilute sulfuric acid. Refluxing the acid solution with barium peroxide and *n*-heptane oxidizes the methadone to benzophenone, which is immediately extracted into the heptane. The heptane layer is removed and washed, and the benzophenone is measured spectrophotometrically. The method is sufficiently sensitive to quantitate therapeutic levels of methadone in small volumes of urine.

Keyphrases □ Methadone in biological specimens—liquid–liquid extraction, barium peroxide–sulfuric acid oxidation, UV analysis □ Barium peroxide–sulfuric acid oxidation—analysis of methadone □ Benzophenone—UV analysis as methadone oxidation product □ UV spectrophotometry—analysis, methadone *via* oxidation to benzophenone

Methadone is an antitussive and analgesic agent (1, 2). In recent years, it has been used effectively as a treatment for narcotic abstinence syndromes (2–4). Several investigators claimed that the methadone maintenence program is the preferred treatment for heroin dependence and reported up to 75% success in achieving sustained rehabilitation (4).

It is apparent that opportunities for abuse of methadone will increase as methadone maintenance programs grow in popularity and number. Gardner (5) reported that the number of deaths resulting from methadone overdose has increased significantly since 1965, the year the maintenance programs were introduced (3). Baden (6), describing a number of deaths resulting from methadone abuse, observed that individuals have obtained large amounts of the drug by enrolling in several programs simultaneously. The increased use of methadone enhances the requirement for its quantitative determination in urine or tissue.

Spectrophotometric methods are available (7, 8), but sensitivity is less than satisfactory. GC and polarographic methods have been described (9–11); however, the degree of technical proficiency required to analyze methadone by these methods makes them unacceptable to many laboratories. This report describes a sensitive spectrophotometric method for determining methadone in biological specimens. Analysis is based upon oxidation of methadone to benzophenone, a compound that has a much greater molar absorptivity for UV radiations than does methadone.

EXPERIMENTAL[1]

Reagents—Methadone hydrochloride USP[2], in appropriate amounts, was dissolved in deionized water. Concentrated sulfuric acid[3] was diluted with water to obtain a 4.7 *M* solution. Barium

[1] A Staco variable autotransformer, Type 2PF1010, was used in conjunction with a CRC Multi-lectric Outlet, model 1035/72, to deliver, on the average, 57 v. to Glas-Col heating mantles. Water-cooled 400-mm. Allihn condensers were attached to 250-ml. flasks positioned in the heating mantles. Magnetic stirrers were positioned beneath the heating mantles to mix the reaction solution and to serve as a support for the entire reflux system. A Beckman DK-2A ratio-recording spectrophotometer with 10-mm. cells was utilized. (Any spectrophotometer capable of accurate measurements at 247 nm. is adequate.)
[2] Mallinckrodt Chemical Co.
[3] Baker analytical reagent.