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Manipulation and magnification of the sweetness of sucrose

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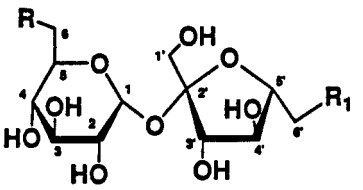
Abstract: Sweetness magnification of sucrose occurs when specific hydroxyls are replaced with chloro groups, particularly at carbons 4, 1', 4' and 6', but not 6, rising progressively from 2 - 20 times in mono-chlorides to >5000 times in tetra-chlorides. This surge in activity is interpreted by the formation of a sweetener-receptor complex, loosely linked by two H-bonds from 2-O ($\mathbf{B}_s$) and 3'-OH ($\mathbf{AH}_s$) of the sucrose derivative to the *N*-asparaginyl unit of an α-helical protein, which is strengthened by dispersive interactions with the side-chains of the receptor protein. These interactions increase in proportion with the degree of chloro-substitution in the sweet compounds, as illustrated by the molecular modelling by computer graphics.

The sweetness of sucrose (**1**, R=R_1=OH), the conventional standard (1x), can be manipulated upwards or downwards in a variety of ways by using either additives or structural changes. Thus, caramel-type molecules, such as maltol or furaneol, enhance its sweetness by 10 - 20% (ref. 1) and so does synergism with H. I. sweeteners, especially aspartame, cyclamate, acesulphame and saccharin. Also, replacement of certain hydroxyl groups in sucrose by chloro groups can either enhance or reduce its sweet taste, the 6-mono-chloride (**1**, R=Cl, R_1=OH) being virtually tasteless whilst the 6'-mono-chloride (**1**, R=OH, R_1=Cl) is 20x sweeter (ref. 2). The presence of arylalkanoic acids, such as *p* -methoxyphenylpropanoic acid, can dramatically reduce the sweetness of sucrose by up to 80%. An understanding of these phenomena requires an initial knowledge of the prosthetic hydroxyls in sucrose that trigger the response, the nature of the taste receptor and its active site, and the mechanism of the interaction with the receptor.

In common with most sweet sugars and polyols, sucrose (**1**) utilises two of its hydroxyls to act as hydrophilic $\mathbf{AH}_s/\mathbf{B}_s$ glucophore, where **A** and **B** are electronegative atoms separated by only 2.5 - 4.0 Å and in this case oxygen, which can interact with a similar $\mathbf{AH}_s/\mathbf{B}_s$ unit on the proteinaceous receptor. In this way a sweetener-receptor complex is formed, joined by two intramolecular H-bonds (**2**) as suggested by Shallenberger and Acree (ref. 3), which allows gauche or staggered α,β–diols to be sweet but not antiperiplaner or eclipsed groups; on a pyranose chair conformation, di-equatorial or equatorial-axial diols are permitted but not trans-diaxial diols. Taste studies on counterparts of each wing of sucrose, namely the slightly sweet methyl α-D-glucopyranoside (0.1x) and the unsweet methyl β-D-fructofuranoside suggested an unusual $\mathbf{AH}_s/\mathbf{B}_s$ unit arising from one hydroxyl on the glucosyl unit and the other on the fructosyl unit (ref. 2). As a further complication, there are two major conformations of sucrose (**1**, R=R_1=OH) in solution, each with an intramolecular H-bond, one between the 1'-OH and 2-O (**3**), and the other from 3'-OH to 2-O (**4**), with the former predominating in the ratio of 2 : 1 (ref. 4). On the above evidence only these pairs of closely placed hydroxyls, at



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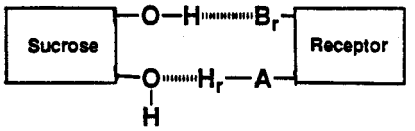

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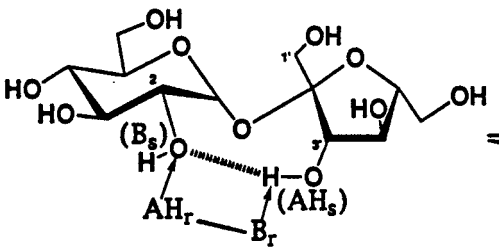

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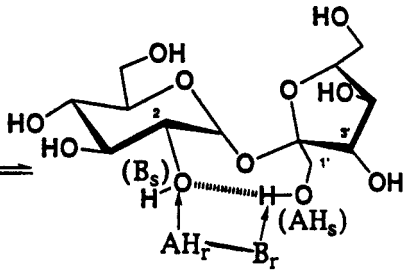

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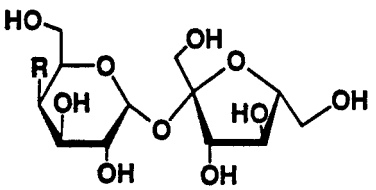

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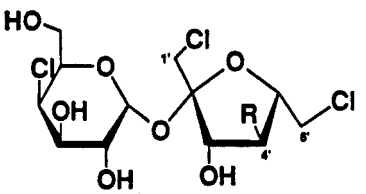

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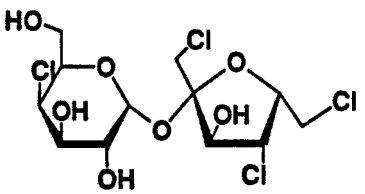

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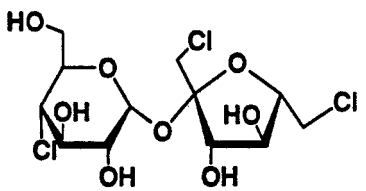

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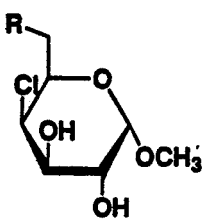

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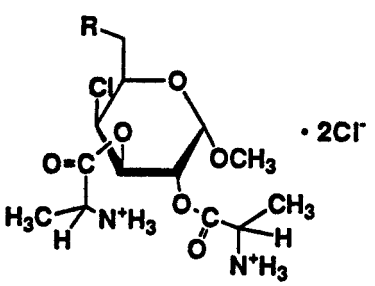

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C-2 and C-3' and at C-2 and C-1', qualify and since 1'-deoxy- and 1'-*O*-methyl-sucrose are sweet, with no 1'-OH, the 2-O ($\mathbf{B_s}$) / 3'-OH ($\mathbf{AH_s}$) is favoured as the major glucophoric trigger. The sweetness of 3,6-anhydro-sucrose also favours this system.

However, the high sweetness of saccharin (>300x) and other high intensity synthetic sweeteners require an additional criteria, as recognised by Kier (ref. 5), and attributed to their lipophilic character in contrast to the hydrophilic, low-sweet sugars. Thus, additional binding to the receptor protein arises via the side-chains of its constituent amino acids and the number and strength of these dispersive interactions ($\mathbf{X_s}$ --- $\mathbf{X_r}$), by van der Waals force etc., determines the intensity of sweetness. Sugars are not very sweet because their hydrophilic hydroxyl groups dominate their lipophilic methylene (-CH_2-) and methine (-CH-) centres. The two methylene groups in fructose (1.8x) do however account for its higher sweetness than glucose (0.6 - 0.75x) and sucrose (1.0x). However, H-bond, dipole-dipole interactions, and van der Waals forces are all weak interactions, with only 1 - 2 Kcal / mole gain on ligand-receptor binding, hence multiple binding is essential for high sweetness, as observed in the cleft of the natural sweet proteins monellin (3000x) and thaumatin (>1600x) (ref. 6 and 7).

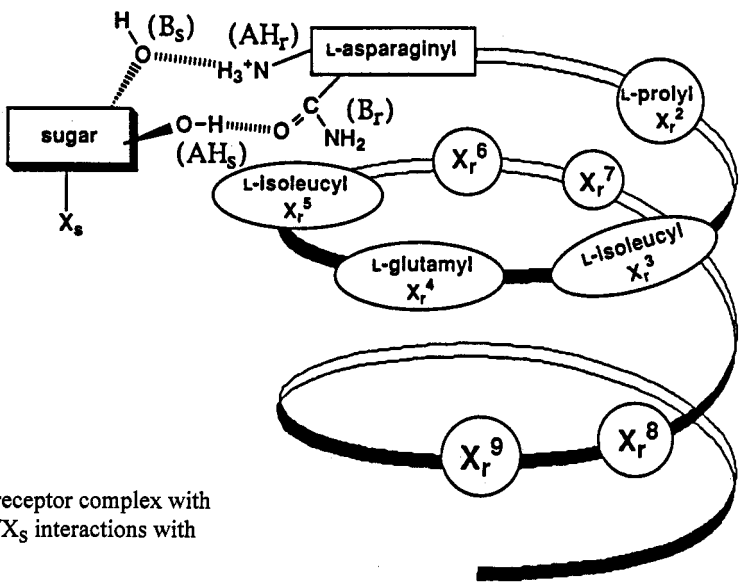

Fig. 1 The sugar–receptor complex with clockwise $AH_S/B_S/X_S$ interactions with $AH_r/B_r/X_r$.

In the absence a characterised receptor protein we proposed (ref. 2) as a model for the **AH / B / X** theory, that an α-helical protein, terminated by an *N*-L-asparaginyl-L-prolyl peptide, followed by L-leucyl units, could account for its sweetness of a diverse range of sweet compounds; subsequent studies suggested an L-glutamyl residue at the fourth position (Fig. 1). Like other neuro-peptide and neuro-transduction receptors, it would be expected to be acting in conjunction with G-protein. Three-point attachment of the tastant, *via* $\mathbf{AH_s / B_s / X_s}$, to the chiral receptor helix, where $\mathbf{AH_r}$ and $\mathbf{B_r}$ reside on the asparaginyl unit at NH_3^+ and O=C-NH_2 respectively, will of necessity, be highly stereo-selective. This docking requires a clockwise arrangement of $\mathbf{AH_s / B_s / X_s}$ when viewed from the active site of the receptor (Fig. 1). Thus D-α-amino acids are sweet whereas L-α-amino acids are not, apart from proline, in contrast to the sugars where D- and L-forms are equi-sweet due to the dual role of the hydroxyl groups, the same two acting as $\mathbf{AH_s / B_s}$ in the D-isomer and as $\mathbf{B_s / AH_s}$ in the L-isomer and both clockwise with $\mathbf{X_s}$ (ref. 2).

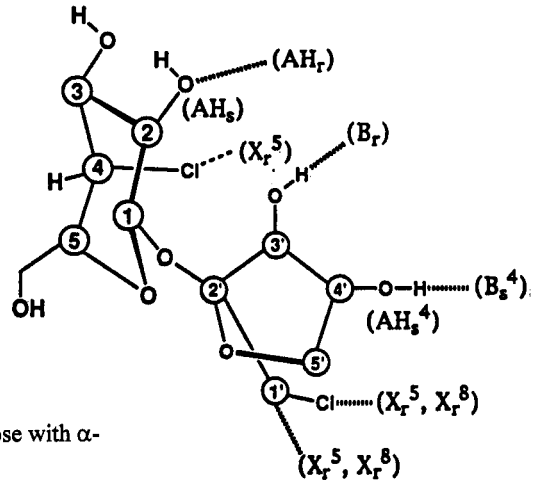

Fig. 2 Interactions of sucralose with α-helical receptor.

Molecular models revealed that both conformations (**4**) of sucrose are capable of interacting with the α-helical receptor, with either 3'-OH or 1'-OH as $\mathbf{AH_s}$, and in each case hydrophobic points (1'-CH_2, 6-CH_2 or 6'-CH_2) on sucrose interacted the protein's side-chains (X_r^4, X_r^5 or X_r^8). Lichtenthaler et. al. (ref. 8) have shown that the hydrophilic surface area of sucrose is centred on the 3-OH of the glucosyl unit, with the hydrophobic area on the opposite half, at the fructosyl 1'-H, 3'-H, 5'-H and 6'-H. Small structural changes at C-4 of sucrose (**1**, R=R_1=OH) revealed interesting taste versus structure results, with *galacto*sucrose (**5**, R=OH) only slightly sweet, 4-deoxysucrose (**5**, R=H) retaining its sweetness and 4-chloro-4-deoxysucrose (**5**, R=Cl) enhanced to 5x. A "4-axial lipophilic effect" explains these results, whereby an axial hydrophilic 4-OH inhibits binding of the 6-CH_2 to X_r^5 (the fifth unit of the receptor), 4-deoxy has little effect, whereas the axial 4-Cl enhances the attraction to X_r^5. Clearly the equatorial 4-OH of sucrose is not essential for sweetness but the lack of sweetness in *allo*sucrose (3-epimer of sucrose) reveals that an equatorial 3-OH is essential (ref. 9).

Similar structural changes at C-6 revealed different results, since the 6-chloro derivative (**1**, R=Cl, R_1=OH) was not sweet, alongside 6-*O*-acetyl, 6-*O*-benzoyl and 6-*O*-benzyl derivatives, whilst 6-*O*-methyl and 6-deoxy derivatives were both sweet, suggesting a "6-steric inhibiting effect". This was confirmed by molecular modelling which showed that larger groups (Cl, CO_2CH_3, CO_2Ph and OC_7H_7) prevented the coupling of $\mathbf{AH_s/B_s}$ to the receptor (ref. 10). Steric inhibition to sweetness was also observed in its unsweet 6,6'-, 4,6- and 1',6-dichlorides whereas in its absence the 6'- and 1'-monochlorides were 4x and 20x sweeter respectively. Hence the sweet taste of sucrose can be quenched by a large substituent at C-6 but magnified by Cl at C-4 (axial), C-1' and C-6' (ref. 2).

When the essential 2-O ($\mathbf{B_s}$) or 3'-OH ($\mathbf{AH_s}$) are absent in sucrose derivatives, as in 2-chloro-2-deoxy-*manno*sucrose (bitter) and 3'-*O*-acetyl-sucrose, sweetness disappears. However a combination of two chloro groups at C-4, C-1', C-4' or C-6' can result in strong synergism, as in the 1',6'-(80x), 4,6'-(50x) and 1',4-(120x) dichlorides but not so pronounced in the 1',4'-(30x) and 4',6'-di-(5x) chlorides. The relatively small enhancement in the two latter derivatives, where 4'-chloro is present, has been attributed to a lack of an additional H-bond ($\mathbf{AH_s^{4'}}$), normally present in sucrose, to a glutamyl unit ($\mathbf{B_r^4}$) at the 4th residue of the protein receptor, thereby reducing the multiple binding.

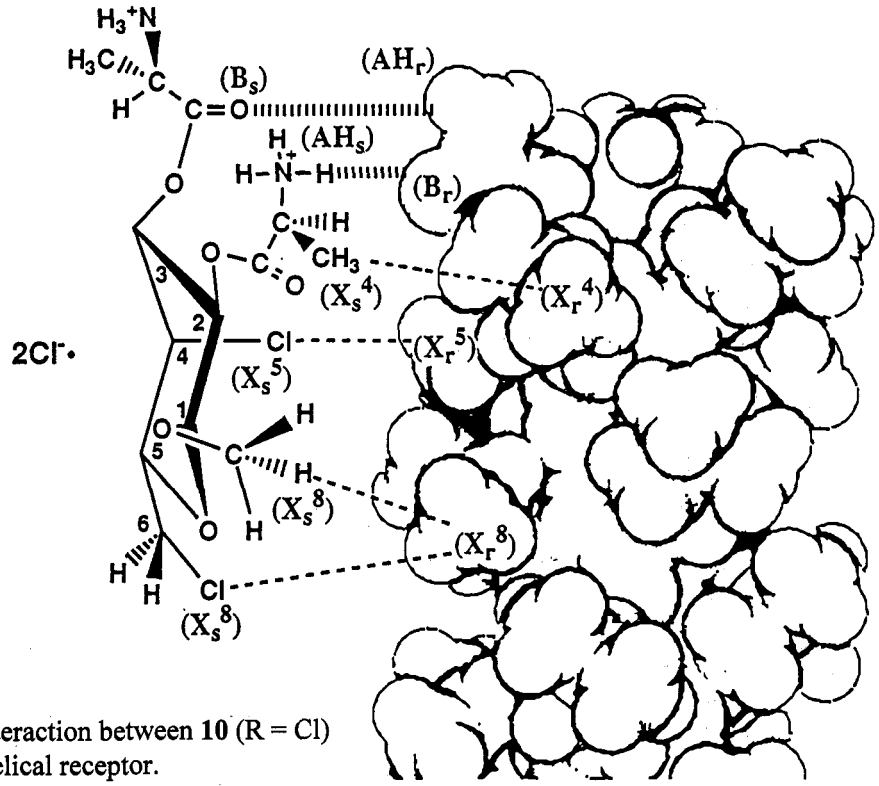

Fig. 3 Interaction between **10** (R = Cl) and the helical receptor.

Further sweetness magnification was observed when three chloro groups were introduced at C-4 (axial), C-1' and C-6' of sucrose, due to even stronger binding to the receptor. This derivative, 4,1',6'-trichloro-4,1',6'-trideoxy-*galacto*sucrose (650x) (**6**, R=OH) has an excellent taste profile, with good solubility in water, more stable than sucrose and is not hydrolysed by invertase or α-glucosidase hence non-caloric and non-cariogenic. Tate and Lyle (U.K.) and Johnson and Johnson (U.S.A.) have developed this product as an H. I. sweetener (ref. 11) currently on the market in several countries as Sucralose and Splenda.

Molecular modelling of sucralose interactions with our α-helical receptor, using 2-*O* / 3'-OH as $\mathbf{AH_s}$ / $\mathbf{B_s}$, revealed contact dispersive attractive forces between 1'-Cl and $\mathbf{X_r}^4$, 1'-CH_2 and $\mathbf{X_r}^5$ and 4-Cl and $\mathbf{X_r}^5$, with an additional H-bond from $\mathbf{AH_s}^{4'}$ to $\mathbf{B_r}^4$ (ref. 12; Fig. 2). The absence of the latter in 4'-deoxy-sucralose (150x) (**6**, R=H) and 4'-*O*-methylsucralose (300x) (**6**, R=OCH_3) resulted in a lower level of sweetness than sucralose (650x). An alternative **AHs / B s** unit, located at 2-OH / 3-O on the glucosyl unit ref. 8 and 13), was also examined by computer generated models of sucralose with the α-helical receptor but they were unfavourable with only one effective interaction, at 4-Cl ($\mathbf{X_s}^4$) and $\mathbf{X_r}^5$. It is also significant that 3,6-anhydro-sucrose is sweet whereas 3,6-3',6'-dianhydro-sucrose is not, neither is sucralose 3',4'-epoxide, lacking the 3'-OH ($\mathbf{AH_s}$), although the latter shows the same dispersive interactions as sucralose with the receptor and hence a possible inhibitor. It is also noteworthy methyl 4-chloro-4-deoxy -α-D-galactopyranoside, a model of half the sucrose molecule, is not sweet and the other half 1,6-dichloro-1',6'-dideoxy-fructofuranose is sweet (1 -2x), but not markedly so.

Further magnification of the sweetness of sucralose was observed when the 4'-OH was replaced by a chloro substituent with retention of configuration, the resulting 1',4,4',6'-tetrachloride (**6**, R=Cl) being 2200x sweeter due to the attractive force between 4'-Cl ($X_s^{4'}$) and X_r^4. This enhancement by halogen is stereoselective since the epimeric 4'-chloro-*sorbo*-isomer (**7**, ref. 12 and 14) was only one tenth as sweet (200x) because the 4'-chloro is remote from X_r^4. For similar reasons the 4-epimer (**8**) of sucralose was also less intense (100x). Clearly the spatial or steric requirements of the hydrophobic regions on sweeteners are highly specific for interaction with their counterparts on the receptor. A relationship between sweetness intensity and the van der Waals radius of 4'-halogen (**6**) derivatives of sucralose has been noted, with F, Cl, Br and I being 1000x, 2200x, 5000x and 7500x as sweet as sucrose due to increasing surface contact of $X_s^{4'}$ with X_r^4 (ref. 2). A similar trend was observed at the 4, 1' and 6' positions, with 4,1',6'-tribromo-4,1',6'-trideoxy-*galacto*-sucrose (800x) and the related 4,1',4',6'-tetrabromide (7500x) being even sweeter but showing no steric inhibition as confirmed by our modelling studies.

The sweetness of sucrose can be manipulated downwards by as much as 80% by inhibitors such as arylalkanoic acids, for example *p*-methoxyphenylpropionic acid (Lactisole), presumably by binding preferentially to the active site ($AH_r / B_r / X_r$) of the receptor, effectively preventing contact with the sweetener. Sweetness inhibitors either lack AH_s units or cannot contact the B_r unit of the receptor for steric reasons. Gymnemic acid and its homologues suppress sweet taste in humans and hydrophobicity plays a major role in this activity (ref. 15). Other inhibitors are methyl 4,6-dichloro-4,6-dideoxy-α-D-galactopyranoside (**9**, R=Cl) (30 - 40% / 0.05M) and methyl 4-chloro-4-deoxy-α-D-galactopyranoside (**9**, R=OH) (<20% / 0.1M), the latter resembling half of the sucralose molecule. However the introduction of an hydrophilic AH_s / B_s unit into these lipophilic inhibitors (**9**) transforms them into sweet compounds such as the 2,3-di-*O*-alanyl derivatives (**10**) which were 75-105x and 36-50x sweeter than sucrose respectively. Another good fit of these alanyl derivatives with the α-helical receptor protein was observed (ref. 2) when the C=O of one alanyl unit positioned as B_s and the other $^+NH_3$ as AH_s, the chloro groups contacting X_r^5 and X_r^8 and the OCH_3 with X_r^8 (Fig. 3). The competitive inhibition of sweetness is consistent with a single site coded for sweet taste on the tongue.

Sucrose shows synergism with various H. I. sweeteners, to give a value higher than expected from the two together such as for examples, aspartame (+11%), cyclamate (+15%) and saccharin (+19%) (ref. 16): also, ethanol shows this enhancement with sugar (ref. 17). A possible explanation is the enlargement of sucrose molecule by inter-molecular H-bonding to the H.I.sweetener, or to ethanol, thereby increasing the number of dispersive sites and hence interaction with the receptor.

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