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Fructose : Structure-Sweetness Relationships on the Basis of Electrostatic and Lipophilicity Potential Profiles

Abstract: Calculations of the **m**olecular **e**lectrostatic **p**otential (**MEP**'s) profiles and of the respective **l**ipophilicity (hydrophobicity) **p**atterns (**MLP**'s) on the contact surface of different fructose conformers were performed. Most informative in regard to the placement of the tripartite AH-B-X glucophore are the hydrophobicity distributions, which show the lipophilic X-part to be an entire, obviously quite flexible region rather than a specific corner of the "sweetness triangle": in fructose the 1- and 6-CH_2 groups contribute almost equally to the most hydrophobic surface regions, while the more hydrophilic part of the 3,4-diol grouping may contain the AH-B couple. Support for this concept is derived from the sweetness data of some fructose derivatives and theoretical considerations. A detailed discussion of the differences between fructose and sorbose based on their MLP's and a comparison of their energetically favorable binding sites is given.

Fructose, the sweetest naturally occurring sugar, is used in pure crystalline form[168] or as high-fructose syrups[169] as a food additive. Despite its widespread utilization, there is still a continuous controversy about the physico-chemical origin of the sweet taste of this compound.

The first rationalization of structure-sweetness relationships by Shallenberger[1,2] and Kier[3] presumes the existence of a common AH-B-X glucophore in all sweet substances, eliciting the sweet response via interaction with a complementary hydrogen bond donor and acceptor functionality and a hydrophobic site in the taste receptor[4,151,152]. This very simple theory, also termed the "sweetness triangle", appears much too simple to explain all of the observations at the present state of knowledge, particularly when bearing in mind that sweet taste chemoreception is mediated by a cascade of complex biochemical processes[5-12] that are little understood at the cellular and molecular level.

Nevertheless, the concept of a tripartite AH-B-X glucophore has had its merits as a unifying criterion and – despite its neglect of three-dimensional shape and volume – proved useful in rationalizing structure-sweetness relationships in so diverse classes of compounds as amino acids, dipeptides, sulfamides (e.g. saccharin and acesulfame), and sugars in particular, most notably the natural sweeteners sucrose and fructose.

The first development of schematic, box-shaped sweetness receptor presentations[170,171] and molecular parameters like apparent molar volumes as conceptual models[172-174] may now be extended by more detailed insights evoking from modern molecular modeling studies. The availability of advanced computer modeling techniques[114], their application to the elucidation of the conformational properties of carbohydrates in vacuum and in solution[13-16], and particularly the possibility of representing various properties on the contact surface of sugars[17-19] has added a new dimension in the visual perception of sugars. Accordingly, not only the electropositive and electronegative areas on the surface of a sugar molecule may be reliably determined by computational methods, but the hydrophilic and hydrophobic regions as well[17-19], which in terms of interactions with the sweet taste receptor are apt to be of high significance.

D-Fructose crystallizes in the β-D-pyranoid form, as evidenced by X-ray[89] and neutron diffraction[90] structural data. Freshly prepared solutions are almost twice as sweet as sucrose (1.8 x)[91,92], but when equilibration of the β-*p*-form to the tautomeric β-*f*-, α-*f*-, and α-*p*-forms (cf. Fig. 2-13 on p. 39) is complete, the solution is only slightly sweeter than one of sucrose of equal w/v-concentration[91]. From this it was inferred that the two furanoid forms are either substantially less sweet than the β-*p* form or devoid of sweet taste altogether[93]. This conclusion is also supported by the parallelism of decrease of both sweetness and the pyranoid compound (in the equilibrium tautomeric mixture[93-95]) on increasing temperature[93,106].

Although the initially high sweetness data for fructose solutions is believed to originate only from the β-pyranoid isomer, this cannot be proved true: according to the mutarotation of fructose in water, the equilibration – even at ambient temperature and neutral pH-values – is very fast and essentially complete within 10 min[93]. It also should be noted that the tautomerization speed increases dramatically under non-neutral[175], physiological conditions which must be considered when focusing onto the biochemical process of sweetness perception. From this, it is probable that actually all sweetness tests on fructose are done with – at least partially – equilibrated solutions, which contain considerable amounts (up to 25%!) of the β-furanoid form too, and that nobody knows the exact sweetness value for the pure β-pyranoid form.

Fructose-sweetness considerations are all based on the β-*p* form, and several assignments for the tripartite AH-B-X glucophore have been advanced: Shallenberger *et al.*[2,93,96], intuitively, Lindley & Birch[97], on the basis of consideration of model compounds[97], and Szarek *et al.*[176] by interpretation of IR-data arrived at the anomeric 2-hydroxyl group and the hydroxymethyl oxygen as

the AH-B couple, respectively (Fig. 2-14 on p. 39, i), with the 6-methylene group acting as hydrophobic binding point X. The inverse assignment (ii) was suggested by Szarek *et al.*[98-100] and by Mathlouthi & Portmann[20], based on calculations of the net atomic charges and the relative basicities of the hydroxyl groups[98,100] and IR-data rationalizations[20]. These findings were also supported by Suami & Hough[177] on inspection of CPK-models of β-D-fructopyranose and a hypothetical sweetness receptor protein helix, additionally proposing the 1-CH_2-group to represent a sweetness enhancing second dispersive center for hydrophobic interactions[177,178].

Interestingly, however, on the basis of intensity-time studies of the sweetness of glucose and fructose that neither showed differences between α- and β-anomers nor in their apparent molar volumes, Birch *et al.*[101], arrived at an entirely different conclusion: the anomeric center of D-fructose may play no direct role in the sweetness response, but rather the 3,4-diol system to which the AH-B glucophore is to be assigned (Fig. 2-14, iii).

It is unlikely that progress can be made on this rather speculative level by further reflections on the data presently available. If at all, it is clear that the three-dimensional molecular shape of the sweet substrates and their respective physico-chemical properties must be taken into consideration to mold a more precise overall picture of the substrate-receptor-interactions involved in sweetness. Starting from these realizations the results obtained from calculation of the molecular electrostatic potential profiles and hydrophobicity patterns of the β-pyranoid form of D-fructose should be discussed in the sequel.

The availability of advanced computer modeling techniques has opened up new possibilities to analyse the conformational properties of carbohydrates. Understanding the solution behavior of the molecules is an essential prerequisite for rational drug design and evaluation of structure-activity relationships like the biochemical mechanisms of sweet taste.

Molecular Geometry of β-D-Fructopyranose

β-D-Fructopyranose adopts the $^2C_5$-conformation in the solid state[89,90], which is also being retained in solution. Since common ball and stick models do not represent the correct steric properties, for each molecule the contact surface[46] (roughly equivalent to the solvent-accessible surface[47], i.e. "how water sees the molecule") was calculated to illustrate the molecular overall shape as depicted in Fig. 4-1 for the crystal structure (**A**).

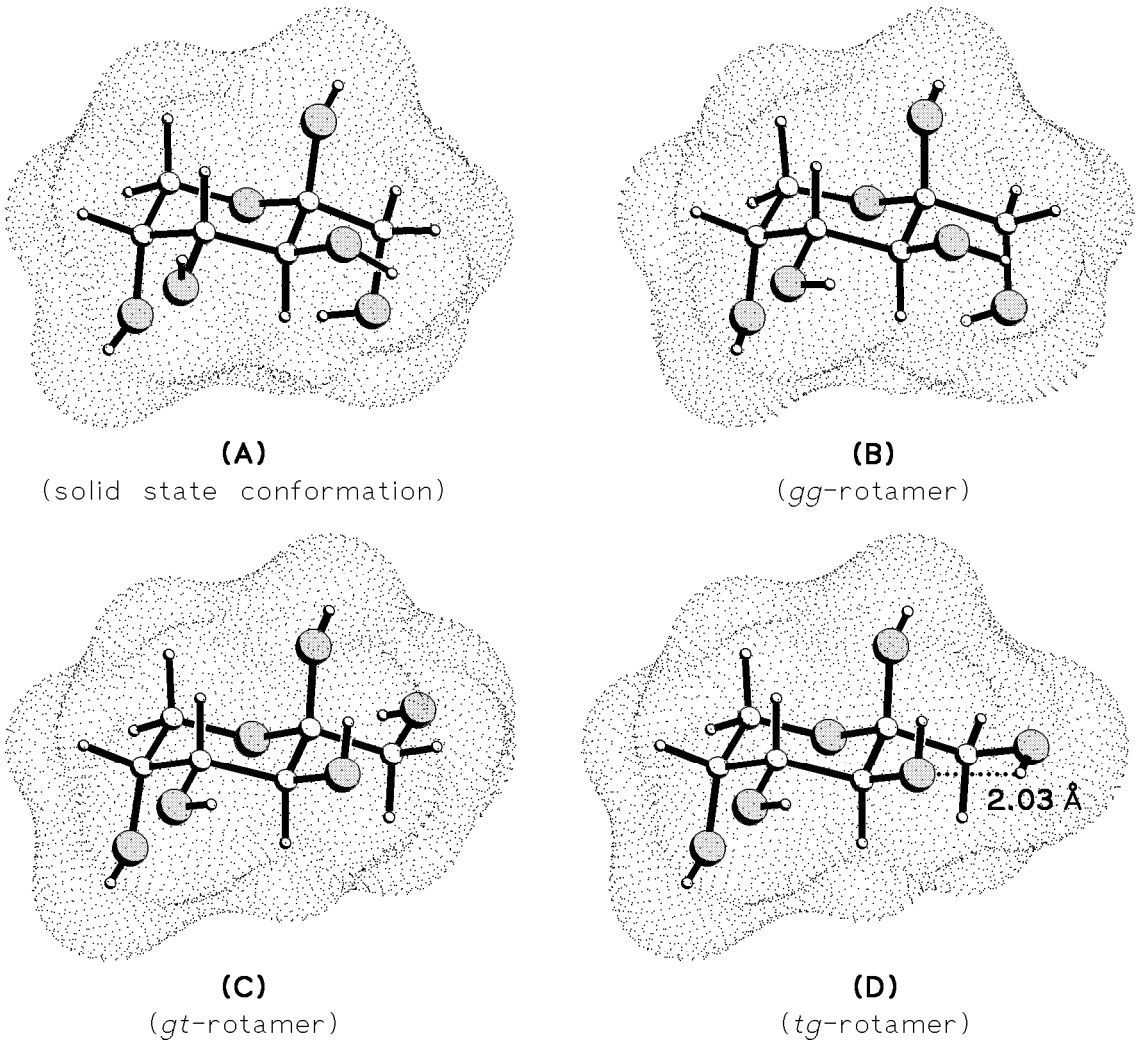


Fig. 4-1. MOLCAD program[48]-generated contact surface of β-D-fructopyranose in dotted form with a ball and stick model insert (oxygen atoms are shaded). (**A**): solid state conformation[89,90]. (**B** – **D**): PIMM88-force field derived, low energy conformers of fructose exhibiting the different *gg*, *gt*, and *tg* arrangements of the hydroxymethyl group[102], respectively. The *tg* rotamer (**D**), despite the unfavorable 1,3-diaxial-like interactions between the 1- and 3-OH group emerges as the lowest energy conformer, due to its stabilization by an intramolecular hydrogen bond 1-OH ··· O-3 (2.03 Å) in vacuo. Since this hydrogen bond will not survive solvation with water, the *tg* rotamer is unlikely to be present in aqueous solutions.

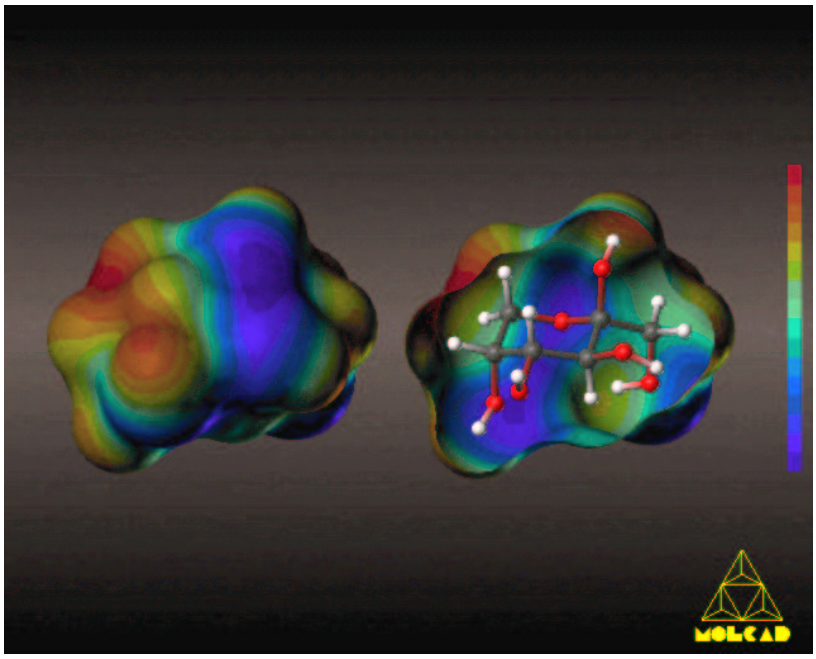
A PIMM88-force field[45] based analysis – focusing especially on the conformational properties of the hydroxymethyl group – revealed the solid state form to represent one low-energy geometry, in which the -CH_2OH unit adopts a *gauche-gauche* (*gg*) arrangement[102] relative to the $^2C_5$-fixed pyranoid ring (PIMM-generated structure **B** in Fig. 4-1, differing from the crystal structure (**A**) mainly by the relaxed torsion angle of the 4-OH group). Undoubtedly, this *gg* rotamer is one form relevant also in aqueous solution, with the minor modification though, that the weak

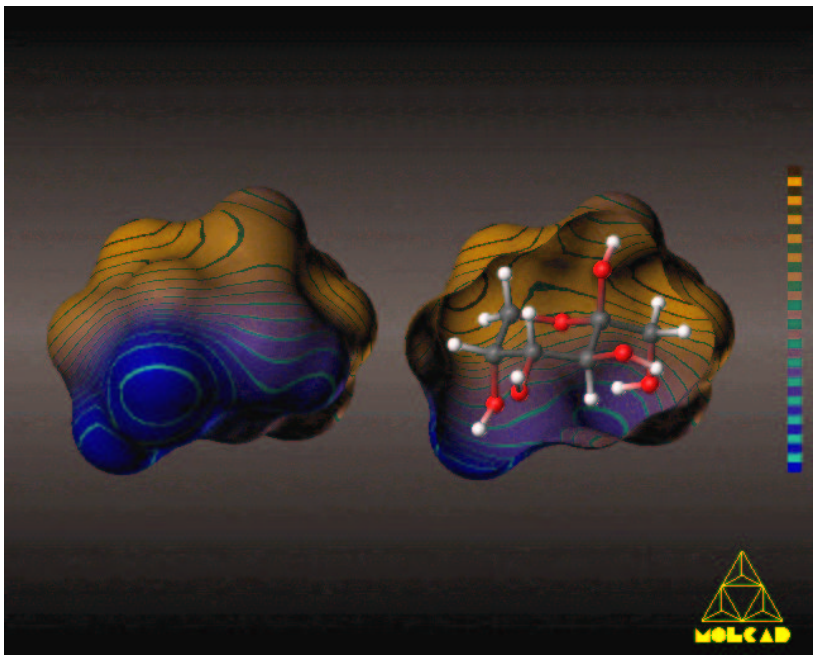
intramolecular hydrogen bond circuit observed in the crystal lattice is disintegrated, since in water the hydroxyl groups can satisfy their hydrogen bond requirements by bonding with the solvent. The *gt* and *tg* rotameric forms of the -CH_2OH group in fructose (**C** and **D** in Fig. 4-1) both represent minimum conformations, too, the latter even being the global minimum energy structure. Calculations of other conformations of fructose are encumbered with the fact that the minimum energy geometries generated represent the state in vacuo, which may substantially be altered on solvation with water, especially when looking at intramolecular hydrogen bonding characteristics. This applies to the conformations emerging from elaborate *ab initio* calculations[98,103] and AM1-based semiempirical[99,100] investigations[179], as well as to those emanating from more simple PIMM88-force field[45] methodology. The *tg* rotamer (**D** in Fig. 4-1), despite steric constraints of the 1,3-diaxial-like arrangement[53,67-73] of the 1-OH and 3-OH group which are overcome (in vacuo) by the stabilizing effect of the intramolecular hydrogen bond 1-OH ··· O-3 (2.03 Å), thus emerges as the most stable form calculated. This situation is most unlikely to prevail in water, particular in view of recent molecular dynamics simulations for methyl β-D-glucopyranoside[72], which convincingly proved the in vacuo minimum energy *tg* form not to survive in water.

This leaves the *gg* and *gt* rotamers of β-D-fructopyranose (**B** and **C** in Fig. 4-1) as the molecular conformations preferred in solution, which have to be entered into a more detailed structural analysis for elucidation of their physico-chemical properties. Certainly, the *gauche*-arrangement of the 1-OH to both the 2-OH group and the ring oxygen additionally favors the *gt* rotamer (**C**) over the *gg* conformer (**A**, **B**), which exhibits an anti-relationship of the 1- and 2-hydroxyl groups.

Molecular Electrostatic Potential (MEP) Profile of β-D-Fructopyranose

The presently most widely accepted assignment for the glucophore of fructose (ii in Fig. 2-14) was proposed on the basis of relative proton affinities and deprotonation enthalpies of the OH-groups, estimated from atomic charges calculated by *ab initio* methods on the STO-3G level[98,103] and semiempirical AM1-methodology[99,100]. Since the calculation of atomic charges is only a rough approximation on the molecular level, the results obtained by different methods may vary substantially – a straightforward interpretation is not possible. Another point is, that the scalar property of point charges completely neglects three-dimensional steric requirements of hydrogen bonding donor- or acceptor capabilities. For these reasons and the experience gained from sucrose[17-19], I think that the electrostatic potential on a molecular surface, which is amenable to interaction with other molecules is more relevant in rationalizing structure-activity-relationships than simple charges are.





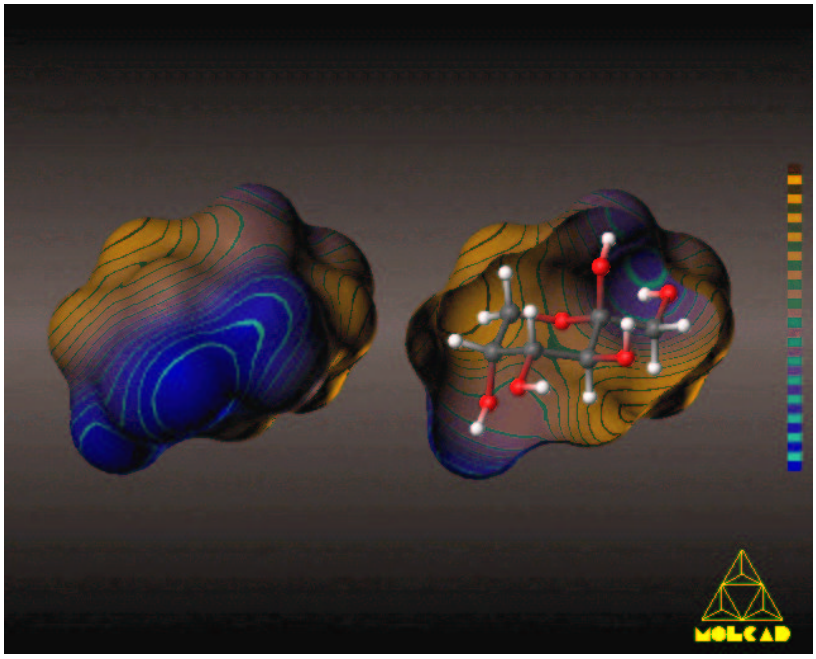


Fig. 4-2. (opposite page, *top* picture). Molecular electrostatic potential (MEP) profile on the contact surface of β-D-fructopyranose, calculated from AM1 atomic charges of the fully energy optimized solid state conformation. Coloring was effected using MOLCAD[48], red areas representing the most positive surface potentials and violet the most negative ones. On the *right*, the surface is partially opened to reveal the back side MEP distribution, the ball and stick model insert providing the molecular orientation.

As outlined above, for generation of the **m**olecular **e**lectrostatic **p**otential (**MEP**)[49] of β-D-fructopyranose the two conformers relevant in solution (*gg* (**B**) and *gt* rotamer (**C**) cf. Fig. 4-1) were used. In both cases the AM1-atomic charges obtained differ to some extent, yet being in good agreement with the *ab initio* charges of Szarek *et al.*[103]. Computation of the MEP on the contact surfaces of both models revealed no qualitative differences (due to the uncertainty of charges in general, I refrain from discussing quantitative effects of small potential changes). Fig. 4-2 represents the MEP of the fully optimized solid state geometry (*gg* conformer) in color-coded form, ranging from red (most positive potential) to violet (most negative potential), the MEP of the *gt* conformer – not depicted here – exhibiting a quite similar overall shape.

A direct assignment of the AH-B unit on the basis of electrostatic potentials is not possible, but it is noteworthy that the electropositive area around the proton of 4-OH and the negative potential on 2-, 3-, and 4-O may make this molecular surface area energetically favorable for hydrogen bonding interactions with a sweet taste receptor. Consequently, these findings – being consistent with Szarek's statement emerging from electrostatic potentials, too, that "the O-4 would be predicted to be the most attractive site for protonation"[103] in fructose – may be interpreted as a hint that the AH-B entity is represented by the 3,4-diol grouping as suggested by Birch[101], rather than the 1,2-diol unit.

Fig. 4-3. (opposite page, *center* and *bottom* picture). MOLCAD-generated[48] molecular lipophilicity pattern (MLP) of the fully energy optimized crystal conformation[89,90] (*gg* rotamer, *center* picture) and the *gt* rotamer (*bottom* picture) of β-D-fructopyranose. For visualization a 32 color code is used, blue representing the most hydrophilic, yellow-brown most hydrophobic regions on the contact surfaces. For unequivocal illustration of the molecular orientation, the half opened forms with a ball and stick model inserted are depicted on the *right* each. While the most lipophilic surface regions of the 1- and 6-CH_2 groups are connected via a "hydrophobic band" in the *gg* conformer, they are separated in the *gt* rotamer.

Another important fact, which is seemingly easily overlooked, encumbers the assessment (ii) in Fig. 2-14 (p. 39), attributing the AH-system to the 1-OH and the B-part to the anomeric hydroxyl group: systematic studies of the hydrogen bonding characteristics in a large number of carbohydrate crystal structures – intramolecular as well as intermolecular in nature – by Jeffrey *et al.*[52,53] revealed that due to the exoanomeric effect anomeric hydroxyl groups are seemingly good hydrogen bond donors, but only very poor acceptors[52,53].

In addition, hydroxyl groups which exhibit only weak hydrogen bond interactions in the crystalline state – like the 1-OH of fructose[89,90] – may have poor hydrogen bonding capabilities in general, making the earlier statement that they are "probably the first to bind to the receptor site when the sugar is dissolved and tasted"[20] questionable.

Molecular Lipophilicity Pattern (MLP) of β-D-Fructopyranose

The interaction of substrates with receptor proteins is not only governed by electrostatic effects – including hydrogen bonding – but also by dispersion forces (van der Waals contacts) and hydrophobic effects[142]. Indeed, sweetness-lipophilicity correlations[160] proved hydrophobic interactions to represent a crucial factor guiding a sweet substrate into the receptor taste bud, and locking it into the correct position for eliciting the sweetness response.

The hydrophobic effect[116,117] may be measured in terms of the partition coefficient of a substance in the water / *n*-octanol system, a molecular property which became calculable by Crippen's development of purely empirical parameters[145]. The latter extension by a distance dependency law opened up the possibility not only to compute the overall coefficient, but also to differentiate hydrophobic and hydrophilic regions – which cannot be easily recognized by simple inspection of CPK models – within a molecule by calculation of the **m**olecular **l**ipophilicity **p**atterns (**MLP**'s) on their surfaces[58,144].

As in the case of the MEP profiles presented above, for generation of the MLP's for β-D-fructopyranose the *gg* and *gt* rotamers of β-D-fructopyranose were used to probe the different AH-B-X glucophore assignments.

Fig. 4-3 depicts the MLP's for both rotamers in color-coded form, respectively, blue representing most hydrophilic, yellow-brown most hydrophobic surface areas. Both forms have their most hydrophilic surface area centered around the fructose-4-OH, whilst the hydrophobic part(s) are associated with either of the two methylene groups: in the *gg* rotamer the two methylene groups are connected with a "hydrophobic band"

that occupies half of the contact surface – as contrasted by the pattern of the *gt* rotamer, where the hydrophobic surface areas of the 1- and 6-CH_2 groups are separated (Fig. 4-3).

Accordingly, the X-part of the tripartite AH-B-X glucophore can easily be located: a region (rather than a specific position) reaching from the 6-CH_2 to the 1-CH_2. The MLP-derived hydrophobic areas of β-D-fructopyranose appear to correlate – at least roughly – with the X-part assignments of Fig. 2-14 that invariably were placed at the 6-CH_2. Since the hydrophobic effect is proportional to the size of the interacting complementary surface areas[148,149], it becomes quite obvious that the hydrophobic interaction of fructose with the sweetness receptor is governed by a large part of the molecular surface (rather than a specific position ("corner") of a "sweetness triangle").

Localization of the AH-B entity on the basis of the MEP's and MLP's is seemingly difficult. Yet, the concentration of the most hydrophilic domain around the fructose-4-OH seems to point to that position for either being the B or AH part, i.e. to the 3,4-diol grouping to represent the AH-B couple. Thus, the MLP's obtained for the two fructose conformers likely to be prevalent in solution favor Birch's[101] proposition (iii in Fig. 2-14), which designates the 3-OH and 4-OH as the AH-B part, respectively.

Experimental Corroboration of AH-B-X-Assignments

Considerations of the few relevant fructose derivatives, whose sweetness characteristics are known, provide no solid evidence with which a clear-cut decision between the putative AH-B-assignments of Fig. 2-14 could be made. That β-D-arabinose (**2**), 2-deoxy-fructose (**3**, 1,5-anhydro-D-mannitol), and the 2-*O*-methyl derivative **4** (methyl β-D-fructopyranoside) are considerably less sweet than the parent fructose[97] advocates the anomeric hydroxyl group to play a role in eliciting sweetness. On the other hand, the fact that sedoheptulosan **5** is as sweet as fructose[104] attests to the contrary.

The sweetness characteristics of analogs **6** – **9** go along with either of the conjectural assignments in Fig. 2-14: the intense sweetness of the 6-*thio* (**6**)[107] and 6-*carba* derivatives (**7**)[108,180] of fructose, although easily rationalized in terms of augmentation of the hydrophobic region within the 6-CH_2-1-CH_2 band, do not allow to differentiate between a 1,2- or 3,4-diol grouping for the AH-B couple of the glucophore. Also, the 5-hydroxyl group can be replaced by hydrogen (→ **8**) without loosing sweetness[105], hence, as such is not an essential requirement for the sweetness sensation. However, its steric (axial) orientation is important since its 5-epimer, α-L-sorbopyranose (**9**), effects a substantial decrease in sweetness[106], possibly by introducing a steric misfit upon interaction with the receptor[105]. Other fructose

derivatives which are suitable to probe the different assignments are either difficult to obtain or their taste characteristics have not reliably been evaluated. 3-*O*-Methyl-β-D-fructopyranose (**10**), for example, was synthesized via 1,2:4,5-di-*O*-isopropyliden-β-D-fructopyranose[181], O-methylation[182], and acetal deprotection[182], and tasted. Evaluation of the taste properties of equilibrated aqueous solutions at 20°C in respect to sucrose and fructose solutions revealed a remarkably lower sweetness. A seemingly straightforward interpretation of this effect in terms of the partial blocking of the 3,4-diol group as the essential AH-B-couple might be misleading: NMR-investigations of the solution equilibrium of different 3-*O*-substituted fructoses like **10** and turanose (3-*O*-α-D-glucopyranosyl-D-fructose) showed a notable shift of the tautomeric mixture composition from the β-pyranoid to the β-furanoid form[93-95], thus being strikingly different from the observed proportions in the case of fructose. In view of the obviously reduced sweetness of the β-furanoid fructose tautomers[93], an explanation of taste effects as in the case of **10** is precarious.

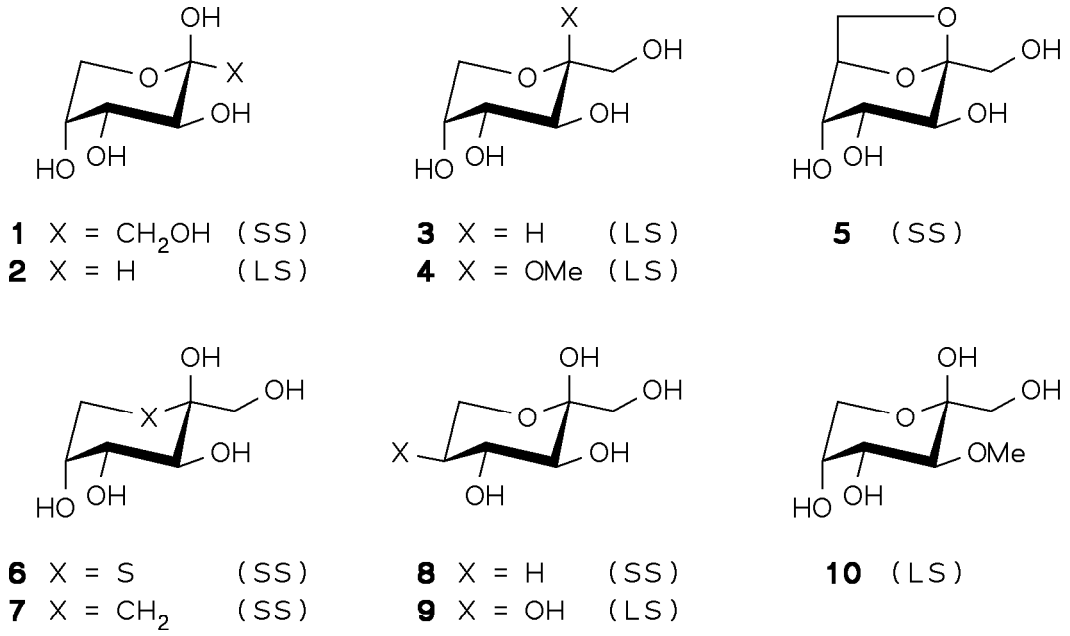
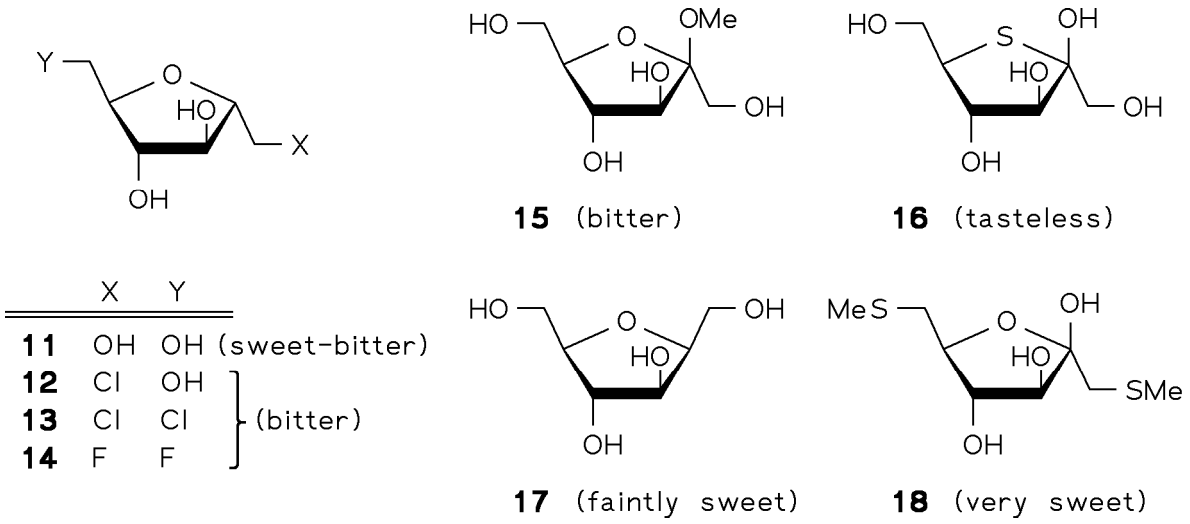


Fig. 4-4. Sweetness characteristics of analogs of β-D-fructopyranose (**1**). (SS: very sweet, LS: low sweetness)

Along with the temperature dependence of the sweetness of fructose and the tautomeric equilibrium, there are some more hints for the lack of flavor of furanoid compounds: 2,5-anhydro-D-mannitol (2-deoxy-β-D-fructo-furanose, **11**)[183], the 1- and / or 6-substituted derivatives **12** – **14**[183], and methyl-β-D-fructo-furanoside (**15**)[93] are reported to exhibit a bitter taste. 5-*Thio*-β-D-fructofuranose **16** is tasteless[107], while 2,5-anhydro-D-glucitol (**17**) has only a faint sweet taste[183]. A

surprising exception in this taste series is made up by the 1,6-di-methylthio derivative **18**, being 15 – 20 times sweeter than sucrose (on a molar basis)[184], an – obviously not trivial – explanation was not provided.



In terms of Shallenberger's AH-B-assignment to the 1,2-diol grouping – which should possess the same steric arrangement in five- and six-membered rings – the lack of taste of the furanoid compounds was explained by the altered stereo geometry of the different rings[93]. The introduction of a "barrier between the usual planar relation between AH, B, and X"[93] by the furanose ring was proposed, yet no assignments of possible hydrophobic binding regions of the five-membered rings were made. Since the molecular geometry of pyranoid and furanoid compounds is quite different, there is no reason to assume that the AH-B-system is to be assigned to the same hydroxyl groups. The straightforward interpretation of the experimental findings is not possible.

The discussion of the sweetness of carbohydrates is always encumbered with the occurrence of considerable amounts of different anomers and tautomeric forms in solution, which might narrow the validity of sweetness evaluation tests. For example, the synthesis and NMR-investigation of 1,6-dihydroxy-2-hexanone ("3,4,5-trideoxy-fructose", **19**) by Szarek *et al.*[185], to probe the AH-B-assignment to the 1,2-diol unit, revealed the presence of the open-chain form as the major compound in solution – due to this reason sweetness tests were not undertaken[185].

Another impeding point is the chirality of the receptor site[170,171]: as evident from the different taste properties of D- and L-amino acids[170,186], one would expect different taste profiles for D- and L-sugars, too. Surprisingly, L-fructose was found to be equally sweet as the naturally occurring D-isomer[170], exciting the discussion of the use of this compound as non-caloric, non-cariogenic[187] (but mild laxative[188])

sweetener (6-*carba*-β-L-fructopyranose was also reported to be almost as sweet as the corresponding mirror image **7**[180], confirming the taste properties of D- and L-fructose).

This obvious contradiction with other chiral substrates was explained with the interchangeability of hydroxyl groups as AH-hydrogen bond donor and B-acceptor systems[170], but due to small substance amounts available and the therefrom resulting uncertainty of the sweetness data, I do not intend to go into further detail on this subject. In particular, the reduced sweetness of L-sorbose (**9**), the 5-epimer of fructose is difficult to understand, and thus, being subject to many speculations so far.

Comparison of β-D-Fructopyranose and α-L-Sorbopyranose

The most striking shortcomings of the proposals (i) and (ii) in Fig. 2-14 for the 1,2-glucophore is seemingly the dramatic difference between fructose and sorbose, being initiated by simple inversion of the remote 5-OH group. The proposed hydrogen bond releasing effect of 5-OH, liberating the anomeric hydroxyl group 2-OH for interaction with the receptor by competitive intramolecular hydrogen bonding with the ring oxygen – being possible in fructose, but not in sorbose – was used to explain the taste difference of these two sugars[97]. Due to the nearly unaltered sweetness of the 5-deoxy derivative **8** in relation to fructose, this explanation seems untenable[105]. In terms of the assignment of 2-OH / 1-O as the AH-B-system (i in Fig. 2-14), a more detailed study by Szarek *et al.*[176] attributed this effect to the general hydrogen bonding capabilities of the individual hydroxyl groups as estimated by correlation with IR-stretching frequencies: in comparison to sorbose, the hydrogen bond donor capacity of the anomeric hydroxyl group of fructose was found to be stronger, thus supporting the enhanced sweetness of fructose[176].

The taste properties of sorbose are more readily rationalized, assuming the validity of Birch's[101] assignment (iii, Fig. 2-14): a change in the steric and electronic characteristics in direct vicinity of the 3,4-diol system would change the sweetness quality substantially. The same effect is observed in the case of sucrose – in which the 2,3-diol unit was assigned as the glucophoric moiety[17-19] – when inverting the configuration at C-4 to "*galacto*-sucrose", sweetness is almost completely lost[63,189]. Similarly, going from glucose (in which the 3- and 4-OH groups seem to represent the AH-B unit[20]) to the 2-epimeric mannose, sweetness changes substantially. Comparing fructose and sorbose, this theory is strongly supported by the molecular lipophilicity pattern of α-L-sorbopyranose, as derived from its crystal structure[190] (Fig. 4-5).

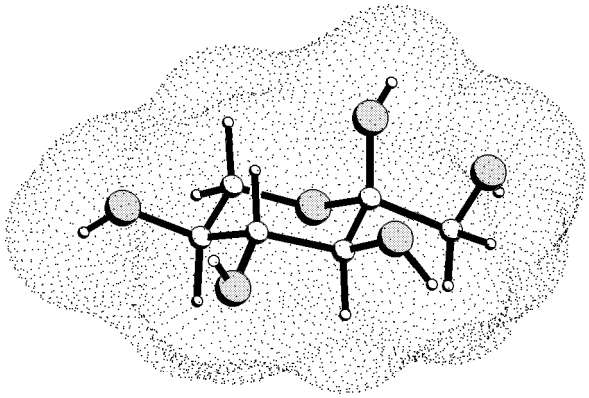


Fig. 4-5. Dotted Connolly surface representation of α-L-sorbose in the crystal[190], featuring the hydroxymethyl group in a *gt* arrangement relative to the pyranoid ring.

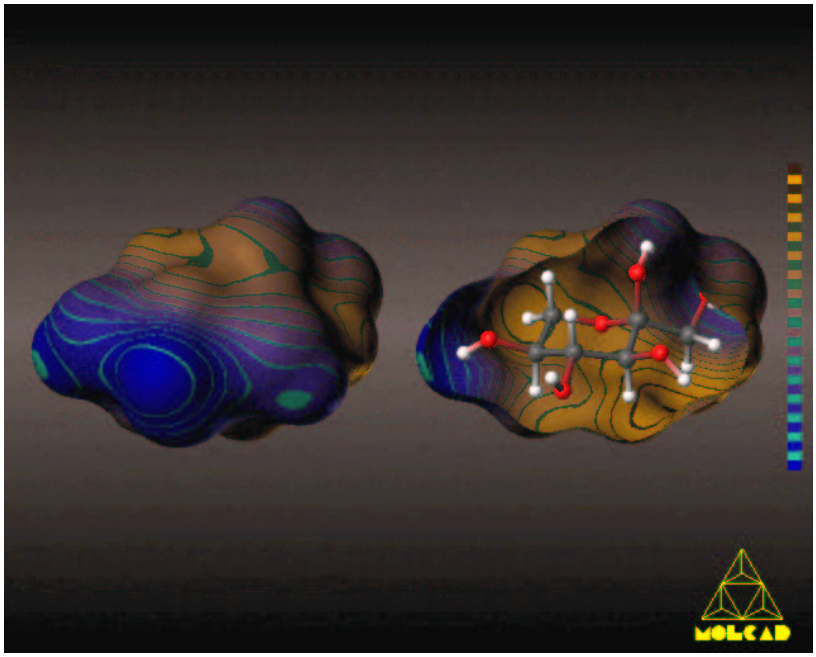


Fig. 4-6. Molecular lipophilicity pattern for the solid state conformation of α-L-sorbopyranose, hydrophilic (blue) and hydrophobic areas (yellow-brown) being visualized in a 32 color-code.

The MLP of sorbose, represented in Fig. 4-6, reveals the most hydrophilic surface region to be spread regularly over the surface of O-3, O-4, and O-5 and, thus, is quite different from the MLP calculated for fructose (cf. Fig. 4-3).

It is now quite understandable that the changed hydrophobicity pattern must alter the binding characteristics towards the receptor, in the case of sorbose obviously leading to a less specific interaction with the sweetness taste bud, and therefore to decreased sweetness. To further probe this hypothesis, the identification of energetically favorable binding sites of fructose and sorbose was tried using the GRID computer program developed by Goodford[191]. Water as a molecule bearing hydrogen

bond donor capabilities as well as acceptor functionalities was chosen as a molecular probe. For a three-dimensional grid the most favorable orientation of a water molecule relative towards the model substance was calculated, assuming free rotation of all hydroxyl groups. The data set obtained allows the calculation of iso-energy contour surfaces, which at negative energy levels delineate regions preferably amenable to receptor interactions.

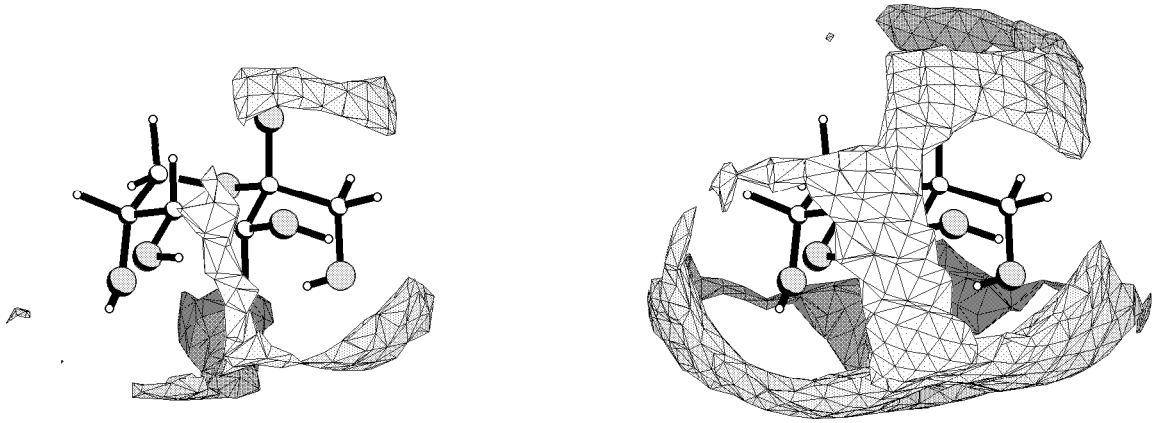


Fig. 4-7. Iso-energy contour plots of the interaction of water with the X-ray-derived molecular geometry of β-D-fructopyranose. The contour surfaces were calculated using the GRID-program[191], assuming free rotation of the hydroxyl groups, and were drawn +3 (*left*) and +4 kcal/mol (*right*) above the global energy minimum of -7.95 kcal/mol (black-and-white shading of the surfaces was carried out to obtain a more lucid three-dimensional picture). Apparently, the 3,4-diol grouping represents a molecular region energetically favored for hydrogen bonding interactions.

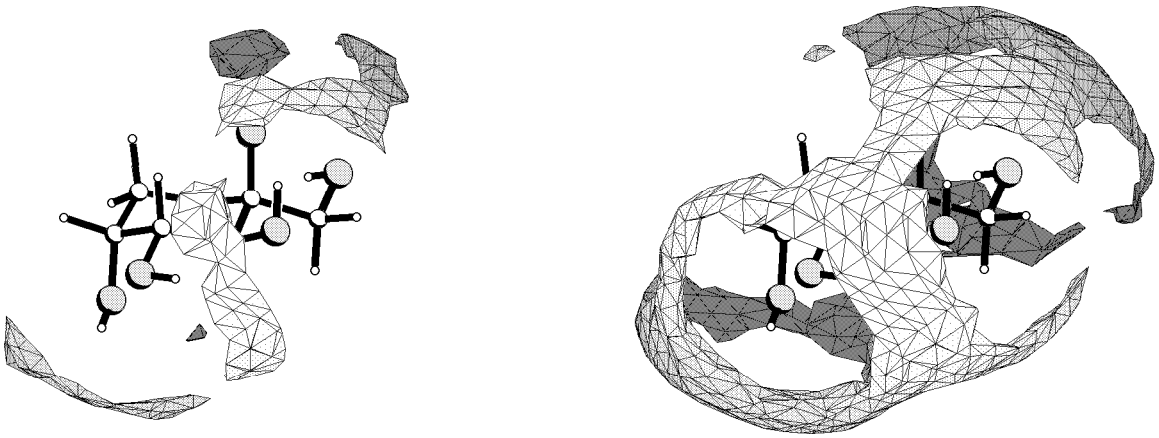


Fig. 4-8. Energy contour plots of the water-fructose interaction at the +1 (*left*) and +2 kcal/mol level (*right*) above the global minimum (-5.82 kcal/mol). In contrast to Fig. 4-7, the PIMM derived molecular geometry of fructose has been used, exhibiting a *gt* arrangement of the hydroxymethyl group.

For the solid state conformation of fructose (*gg* conformation of the CH_2OH-group) as well as for the *gt* rotameric form, Fig. 4-7 and 4-8 additionally confirm the theory developed above: the 3,4-diol unit most probably represents the AH-B glucophoric system. Investigation of the *tg* form (**B** in Fig. 4-1) and other molecular geometries – like the global minimum energy form proposed by Szarek *et al.*[99] – showed analogous trends. The same calculation for L-sorbose (Fig. 4-9) shows more unspecific interactions as widely spread regions, which most remarkably not only comprise the 3- and 4-OH groups, but also the 4,5-diol grouping. This was already stated on the basis of the MLP's (Fig. 4-6).

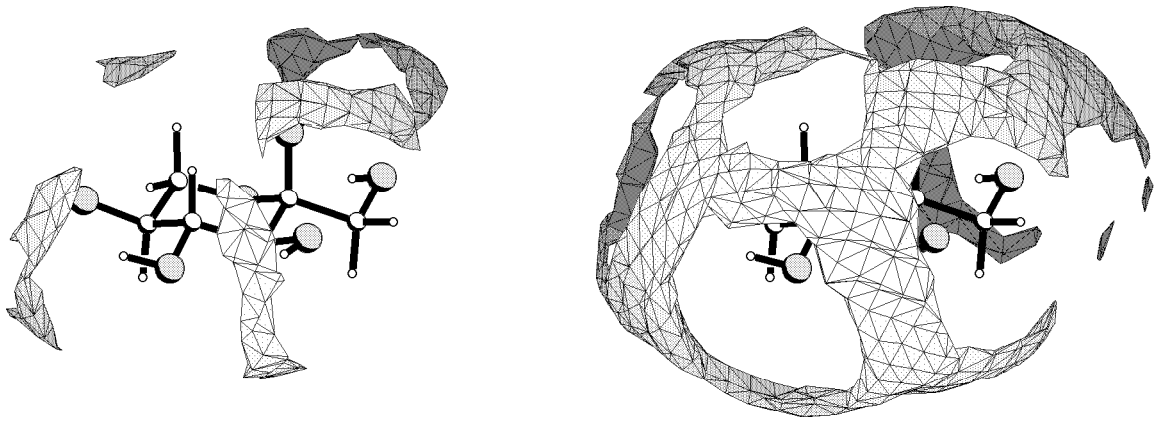


Fig. 4-9. Iso-energy contour surfaces of the interaction of α-L-sorbose in its crystal conformation with water were calculated at the +1 and +2 kcal/mol level above the absolute minimum of -5.90 kcal/mol. Most remarkably, the energetically favorable binding sites are not only located at the 3,4-diol grouping (as in fructose, cf. Fig. 4-8 and 4-9), but also at the hydroxyl groups in position 4 and 5. This extended, more unspecific region may lead to a misfit upon interaction with the sweet taste receptor, explaining the dramatically decreased sweetness of sorbose as compared to fructose.

These qualitative differences are not only present when using water as a molecular model to probe into possible binding sites: both carboxylate anions ($-COO^-$, hydrogen bond acceptor only) as well as ammonium cations ($-NH_3^+$, donor only) – not depicted here – seem to exhibit favorable interactions with the 3,4-diol units of fructose. In sorbose, those regions become more extended and unspecific, as would have been expected from the results mentioned above.

It is also important to mention that the *gg* conformer of fructose exhibits more stable binding characteristics (approx. -8 kcal/mol) than sorbose does (-5.9 kcal/mol), being consistent with a more specific interaction and therefore the enhanced sweetness of fructose.

Although further evidence is required to settle this question unequivocally, as of now, major significance is attributed to the MLP's obtained for the two fructose conformers likely to prevail in solution: these (Fig. 4-3) clearly favor Birch's proposal[101] (iii in Fig. 2-14), which places the AH-B couple of the glucophore into the 3,4-diol grouping of fructose.

Conclusions

When focusing on the essentials contained in the MLP's of the two fructose forms prevalent in solution, the basic feature emerges that hydrophobic and hydrophilic regions are located on opposite sides of the molecule. Thus, it may well be – and this receives fortification from the lipophilicity patterns of sucrose and a number of non-carbohydrate sweeteners[17-19] – that the distribution of hydrophobic and hydrophilic regions on opposite sides, the latter being capable for hydrogen bonding with the receptor, is a principal structural feature for eliciting sweetness, and not necessarily the modified AH-B-X "sweetness triangle".

The sweet receptor – be it the same for sucrose, fructose, and non-carbohydrate sweeteners or not – is seemingly quite flexible in adapting to the hydrophobic region of sweet substances, to the X part quasi (of the tripartite AH-B-X glucophore), which clearly is not a specific position of the molecule, but an entire region. The hydrophobic area is seemingly the main factor governing the "docking procedure" of the sweet substance, i.e. directing it to and locking it into the complementary "hydrophobic cleft" of the receptor protein. The opposite hydrophilic area of the molecule, containing the AH-B portion of the Shallenberger-Kier tripartite AH-B-X glucophore, is brought into the appropriate position to elicit the sweet response via hydrogen bonding to a complementary receptor site AH-B couple.

In summary, much remains to be learned about the intricacies of the mechanism(s) involved in activation of sweet-sensitive cells, and direct solid evidence is urgently required. Nevertheless, the incorporation of the three-dimensional shape of sweet molecules, of their contact surfaces, and, particularly, inclusion of their MEP's and MLP's into structure-sweetness considerations has provided this field with new dynamics, not only in the visualization of the sweet molecule as such, but also of the complementary binding site. This unfoldment is apt to lead, via computer-aided receptor modeling, to more realistic structure-sweetness concepts than those developed before.

Appendix – Computational Methods

Crystal structures were retrieven from the Cambridge crystallographic database[192]. The conformational analysis of fructopyranose was carried out by the force field program PIMM88[45], followed by an AM1[51] opimization of selected most stable structures applying the keyword "PRECISE". The molecular modeling software package MOLCAD[48] was used for the calculation, coloring, and representation of the contact surfaces[46] and the corresponding molecular electrostatic potential profiles[49] and hydrophobicity patterns[58] (for details see Chapter 3). The MEP patterns are based on the atomic charges as obtained after full geometry optimization on the AM1[51] level using MOPAC[50]. Iso-energy surfaces were calculated using the GRID program[191] as interfaced by MOLCAD[48].