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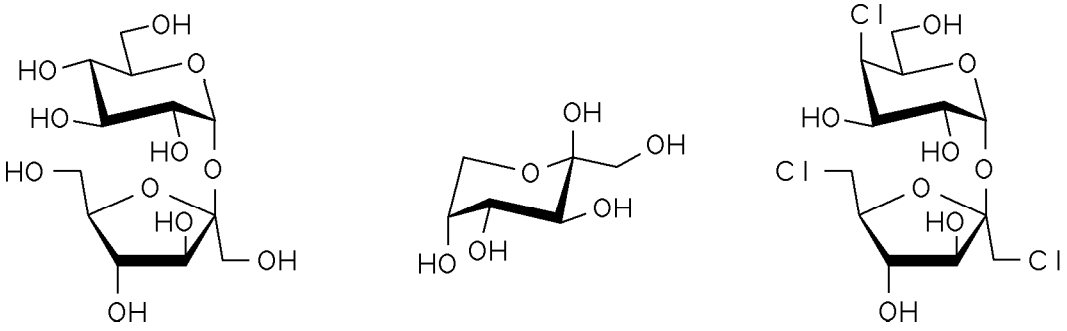
Sucrose, Sucralose, Fructose, and some Non-Carbohydrate High-Potency Sweeteners : Correlations Between Hydrophobicity Patterns and AH-B-X Assignments

Abstract: MOLCAD program-mediated 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 surfaces of sucrose, *galacto*-sucrose, sucralose, and fructose are represented in color-coded form. Most informative with respect 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 sucrose and sucralose encompassing the outside area of the fructofuranose moiety, in fructose the 1- and 6-CH_2 groups in either linked or separated form. In contrast, the hydrophilic portions of these sweeteners are more compact, invariably located opposite to the hydrophobic region, and appear to contain the AH-B couple of the glucophore: the glucosyl-2- and 3-OH group in sucrose and sucralose, versus the 3,4-diol grouping in fructose. Whilst absolute proof for these assignments is still lacking, support for their relevance is derived from the sweetness of altogether 53 sucrose derivatives and some fructose analogs. Most remarkably, the MLP's generated for the solid state conformations of some non-carbohydrate high-potency sweeteners, such as the sulfamides cyclamate, saccharin, and acesulfame, as well as structurally distinctly different dipeptides, e.g. aspartame, exhibit a hydrophobicity distribution strikingly similar to those observed for the sugars: hydrophilic and hydrophobic areas on opposite sites of the molecule. The results, particularly the lipophilicity patterns presented, sustain the notion, that the sweet receptor with its proteinaceous "hydrophobic cleft" – be it the same for sucrose, fructose, and non-carbohydrate sweeteners or different ones – is quite flexible in adapting to the complementary hydrophobic region of the sweet substance. Following this "docking procedure", the hydrophilic AH-B area of the substrate now being in its proper position, the sweet response is elicited via hydrogen bonding to a complementary receptor-based AH-B couple.

The classical attempt by Shallenberger[1,2] and Kier[3] to rationalize the sweet taste of organic compounds presumes the existence of a common AH-B-X glucophore in all sweet substances, eliciting the sweet response via interaction with a complementary tripartite AH-B-X site in the taste bud receptor[4].

At the present state of knowledge, however, this "sweetness triangle" appears much too simple to explain all of the observations, particularly when bearing in mind that sweet taste perception is mediated by a cascade of complex biochemical processes[5-12] that are little understood at the cellular and molecular level.

Nevertheless, the tripartite AH-B-X glucophore concept has had its merits as a unifying criterion and proved useful – despite its neglect of three-dimensional shape and volume – in rationalizing structure-sweetness relationships in such diverse classes of compounds as amino acids, dipeptides, sulfamides (e.g. saccharin and acesulfame), and sugars in particular, most notably the natural sweeteners sucrose (**1**) and fructose (**2**), as well as sucralose (**3**), a sucrose-derived high-potency sweetener.



sucrose **1** β-D-fructose **2** sucralose **3**

The availability of advanced computer modeling techniques, their application to the elucidation of the individual conformations of carbohydrates in the vacuum and in solution[13-16], 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 may the electropositive and electronegative areas on the surface of a sugar molecule 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 great significance.

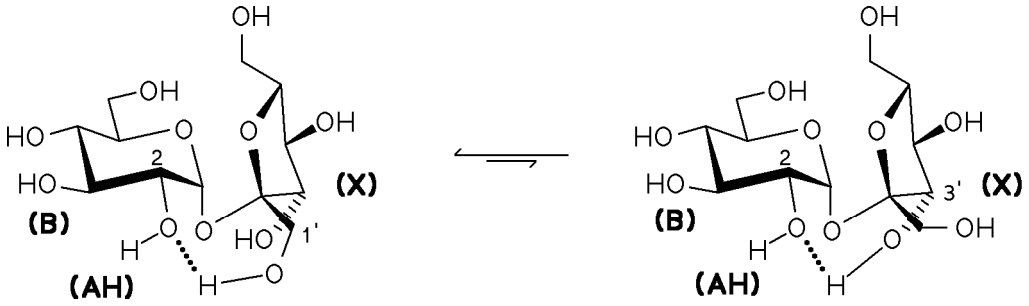


Fig. 2-1. Location of the tripartite AH-B-X glucophore in sucrose emerging from the computer-generated molecular electrostatic potential (MEP's) profiles and lipophilicity patterns (MLP's)[17-19].

Incorporation of such results into structure-sweetness considerations have led to a new allocation of the Shallenberger-Kier[1-3] tripartite AH-B-X glucophore in sucrose for the two forms likely to prevail in solution: the glucosyl-2-OH being the H-donor in this hydrogen bond interaction with a complementary acceptor group in the receptor, entailing the glucosyl-3-OH as the H-acceptor (B site), whilst the hydrophobic X-part is an area on the "outside" of the fructose moiety (Fig. 2-1).

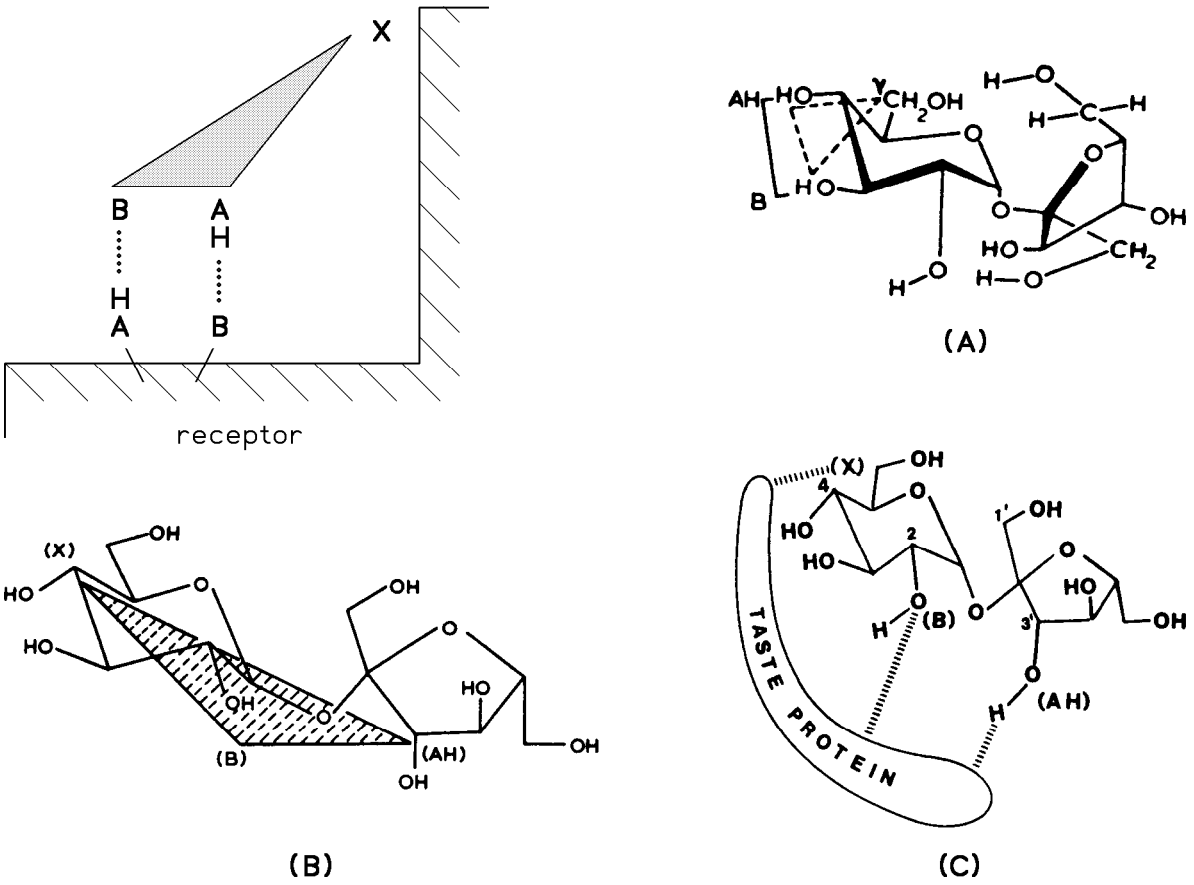


Fig. 2-2. Shallenberger / Kier[1-3] concept of structure sweetness relationships which was assessed by Lee[4] "to be devoid of any predictive value": a hydrogen bond donor (AH), a hydrogen bond acceptor functionality (B), and an additional hydrophobic binding point (X) are considered to be the essential structural element of all sweet tasting molecules. For sucrose, different AH-B-X-assignments have been proposed by Mathlouthi *et al.* in 1990 (**A**[20]) and by Hough *et al.* in 1989 (**B**[21] and **C**[22]).

Whilst these conclusions derived from the contact surface distribution of the molecular electrostatic potential (MEP's) profiles and the molecular lipophilicity (hydrophobicity) patterns (MLP's) of sucrose appear to be reasonable, they differ from alternative AH-B-X assignments as in Fig. 2-2, which are based on rationalizations of the sweetness of a sizeable number of sucrose analogs. As indicated in Fig. 2-2, the hydrophobic center X is placed at the axial 4-position of the glucose moiety, because

of the lack of sweetness of *galacto*-sucrose (the 4-epimer of sucrose), the sweetness of 4-deoxy-sucrose (1 x), and the enhanced sweetness of 4-chloro-4-deoxy-*galacto*-sucrose (5 x)[21-23]. However, alternative hydrophobic centers had to be placed at the 1'- and 6'-positions of the fructose unit to account for the increased sweetness (20 x) of the respective 1'- and 6'-chloro-deoxy-sucroses[21-23]. In terms of the early suggestion of Shallenberger[24] that the presence of hydrophilic centers at different parts of the molecule may drastically disturb their orientation pattern, thereby preventing interactions of the AH-B units with the receptor site(s), multiple centers of hydrophobicity located in different portions of the sweetener appear to be unlikely.

In view of the fairly reliable MEP's and MLP's on which the assignment in Fig. 2-1 is based, it appeared imperative to subject all sucrose derivatives of which the sweetness characteristics are known, to a thorough scrutiny with respect to the validity of the various AH-B-X assignments. This is done in the sequel based on the sweetness characteristics of deoxy-, *O*-methyl and deoxy-halo derivatives of sucrose. In addition, the hydrophobicity patterns of fructose (**2**), in its β-pyranoid form, and some non-carbohydrate sweeteners are probed as to their implications for the AH-B-X concept.

The Electrostatic Potential Profiles and Hydrophobicity Patterns of Sucrose

As evidenced by solid state structural data[25,26], the conformation of sucrose in the crystalline state is determined by two intramolecular hydrogen bonds, one between the primary 6'-OH of fructose and the pyranoid ring oxygen of glucose (1.89 Å, cf. Fig. 2-3**A**, and ball and stick model in Fig. 2-4**A**), the other one between the 1'-hydroxyl group and the 2-O of glucose (1.85 Å). In solution, however, particularly in water, it is unlikely that both of these hydrogen bonds are retained. Indeed, elaborate NMR investigations[27-37] strongly attest to the disintegration of the weaker $5\text{-O}^{g} \cdots \text{HO-6}^{f}$ hydrogen bond by solvation. For a dimethyl sulfoxide solution a competitive equilibrium between forms **B** and **C** has been deduced[30], with the former predominating.

Several calculations of the energy potential surface of sucrose[29,38-44] have provided additional indications concerning the relevance of forms **B** and **C**, including ours using the PIMM88 force field program[45] (see this work, Chapter 3). In Fig. 2-4, the solid state conformation of sucrose (**A**) is set against its PIMM-generated, lowest energy conformation **B**, in which the remaining intramolecular hydrogen bond has been slightly widened to 2.00 Å. For this conformation – being a realistic model for sucrose in (aqueous) solution, and hence, for the form entering into the receptor site – the contact surface[46] (roughly equivalent to the solvent-accessible surface[47],

i.e. "how water sees the molecule") was calculated using the MOLCAD-program methodology[48]. As evident from the dotted contours in Fig. 2-4, the transition from the solid state conformation (**A**) to that likely to prevail in solution (**B**) results only in minor changes on the "upper" side of the molecule.

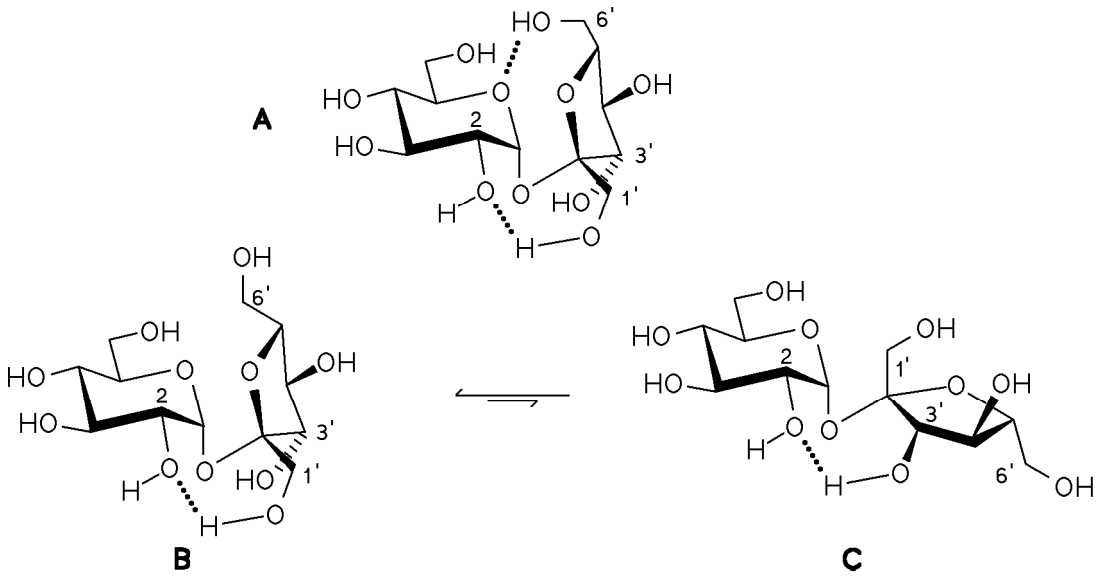


Fig. 2-3. Conformations of sucrose: (**A**) in the crystal as derived from neutron diffraction data[25]. For DMSO solution a competitive equilibrium **B** ↔ **C**, with **B** predominating has been proposed[30].

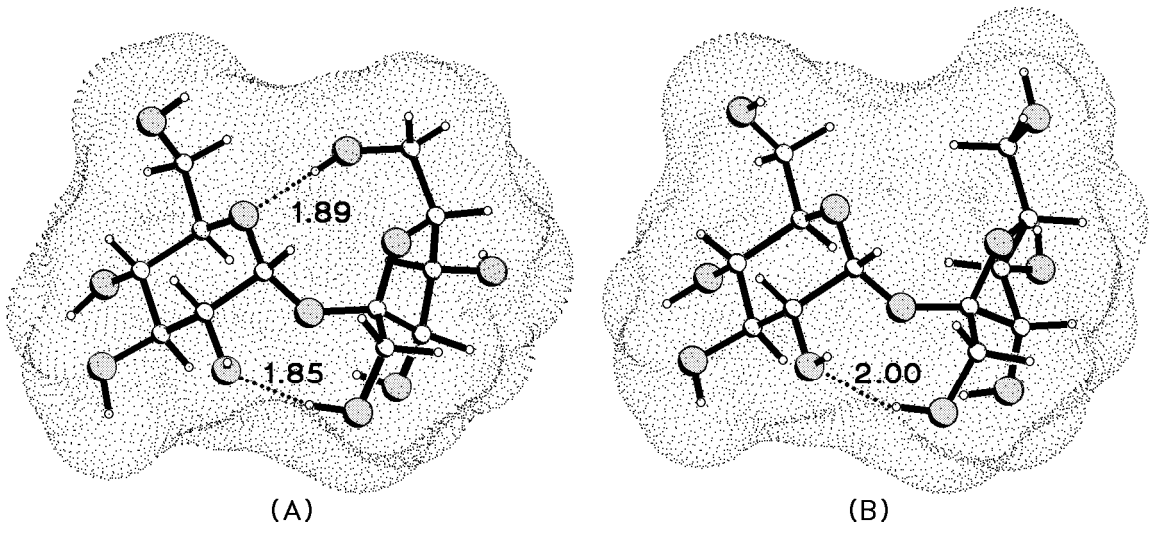


Fig. 2-4. Contact surface (roughly equivalent to the solvent accessible surface) of sucrose in dotted form with a stick-ball model insert, the larger balls representing oxygen atoms. (**A**): conformation in the crystal as derived from neutron diffraction data[25], showing the two intramolecular hydrogen bonds 5-O^{g} ··· HO-6^{f} (1.89 Å) and 2-O^{g} ··· HO-1^{f} (1.85 Å). (**B**): lowest energy conformation emerging from PIMM88 force field calculations (for vacuum), lacking the 5-O^{g} ··· HO-6^{f} hydrogen bond.

Calculations of the molecular electrostatic potential[49] (MEP), i.e. the distribution of the charge density over the contact surfaces of the two sucrose conformations, were effected using the MOPAC[50] program-generated AM1[51]-atomic charges and are represented in a 16 color-code ranging from red (electropositive) to violet (electronegative, cf. Fig. 2-5). These reveal only minor differences in the overall electrostatic profile.

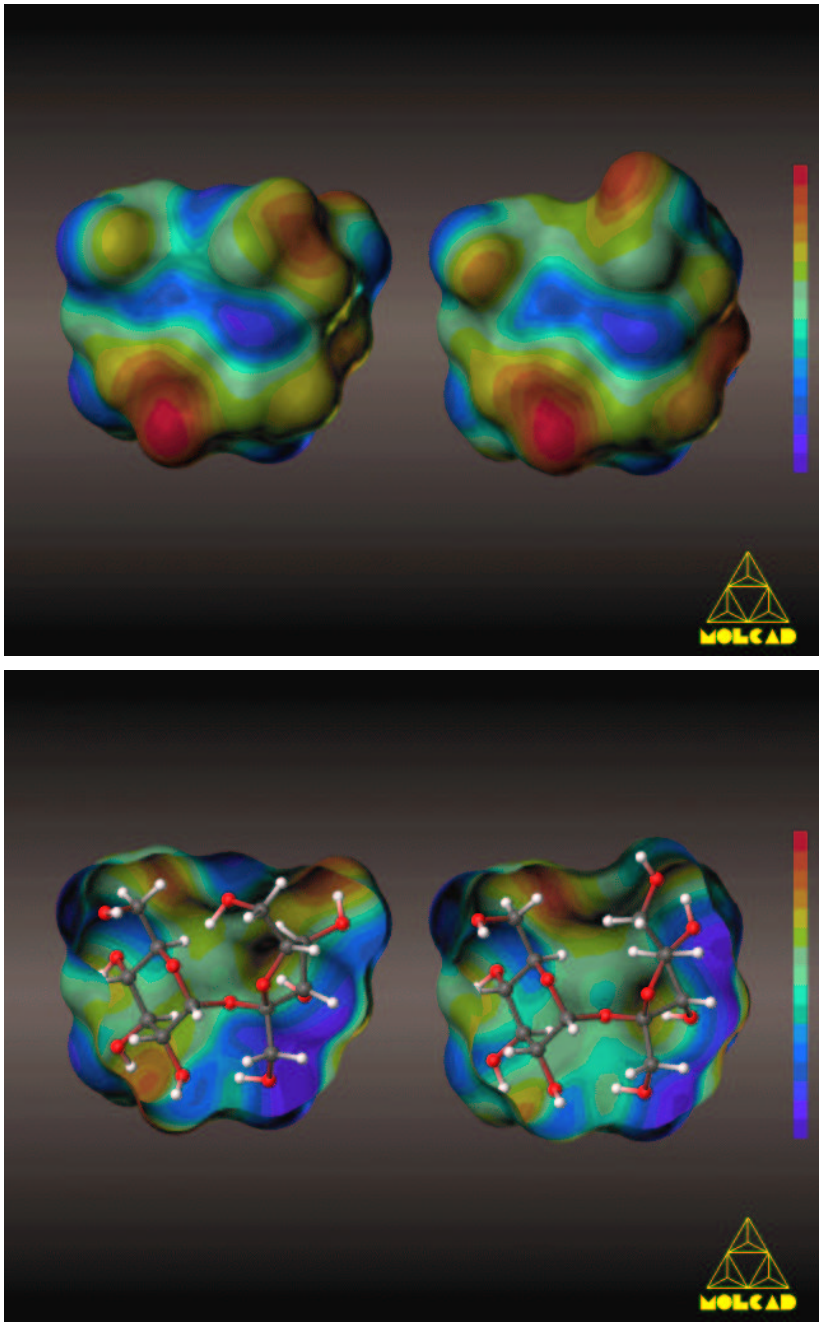


Fig. 2-5. Molecular electrostatic potential (MEP) of sucrose in its conformation adopted in the crystal[25] (closed and opened form with ball and stick model insert on the *left* each), and its most probable "solvated" form in solution (*right* each), represented on the respective contact surface in a 16 color code, red representing the positive maximum, i.e. the most electropositive potentials, violet the most negative portion of the molecule.

As is clearly apparent from Fig. 2-5, each of the forms has the red, i.e. most electropositive area centered around the glucosyl-2-OH, which in turn appears to be caused by the cooperative effect[52-54] of the intramolecular hydrogen bond directed towards the oxygen of this hydroxyl group. In this context, it may be noted that an experimental verification of the validity of the MEP distribution of Fig. 2-5 is provided by the behavior of DMF solutions of sucrose under electrolysis conditions[55]: the molecule is attracted to the cathode with its most electropositive, i.e. the glucosyl-2-OH portion, and upon taking up an electron (to an alkoxyl radical anion) and releasing a hydrogen atom ($\rightarrow H_2$)[56] leaves the glucosyl-2-oxygen deprotonated. This is evidenced by the fact, that in situ alkylation or acylation (i.e. in the cathodic cell) produces the 2-*O*-substituted sucrose derivatives with high preference[57].

The MEP pattern of sucrose as depicted in Fig. 2-5 may thus be considered, with confidence, to properly represent the charge distribution in solution, and, accordingly, may be used to locate the AH-B part of the glucophore such that the AH-hydrogen is likely to be electropositive for interaction with an oppositely polarized binding site on the receptor. Similarly, the inverse situation would have to prevail for the B portion. This reflection points towards the glucosyl-2-OH as the AH portion and the B part in its direct vicinity. However, it is seemingly unavailing to locate the hydrophobic X-site on the basis of the MEP pattern.

Since there is ample evidence to assume that the sweet-taste receptor is proteinaceous in nature, and that interaction between a hydrophobic portion on the protein surface with the corresponding hydrophobic portion of sucrose is involved in triggering the sweet-response, the reliable location of that is of major importance in structure-sweetness considerations. The recently advanced possibility to compute and visualize molecular hydrophobicity (lipophilicity) patterns (MLP's)[58] was applied[17] to determine the lipophilicity profile for crystalline sucrose as depicted on the left of Fig. 2-6 in an alternative 32 color-code[59], blue representing here the most hydrophilic and yellow-brown the respective most hydrophobic areas. As is clearly evident from this representation, the hydrophilic and hydrophobic portions of the molecule are distinctly separated on opposite sides. Particularly lucid is the half-opened form with the ball and stick-model insert, disclosing the entire outside-section of the fructose moiety to be hydrophobic (i.e. yellow-brown), and the hydrophilic (blue) section to be centered around the 3-oxygen of glucose[17].

Observation of the alternative sucrose conformation likely to prevail in solution (cf. Fig. 2-6, right), reveals few changes in the overall MLP, except that the hydrophobic region located at the outer side of the fructose moiety is now more compact. In consequence, the part of sucrose amenable to engage in hydrophobic

bonding within the sweet-taste receptor is to be assigned to an entire region in the fructose portion, rather than a specific position.

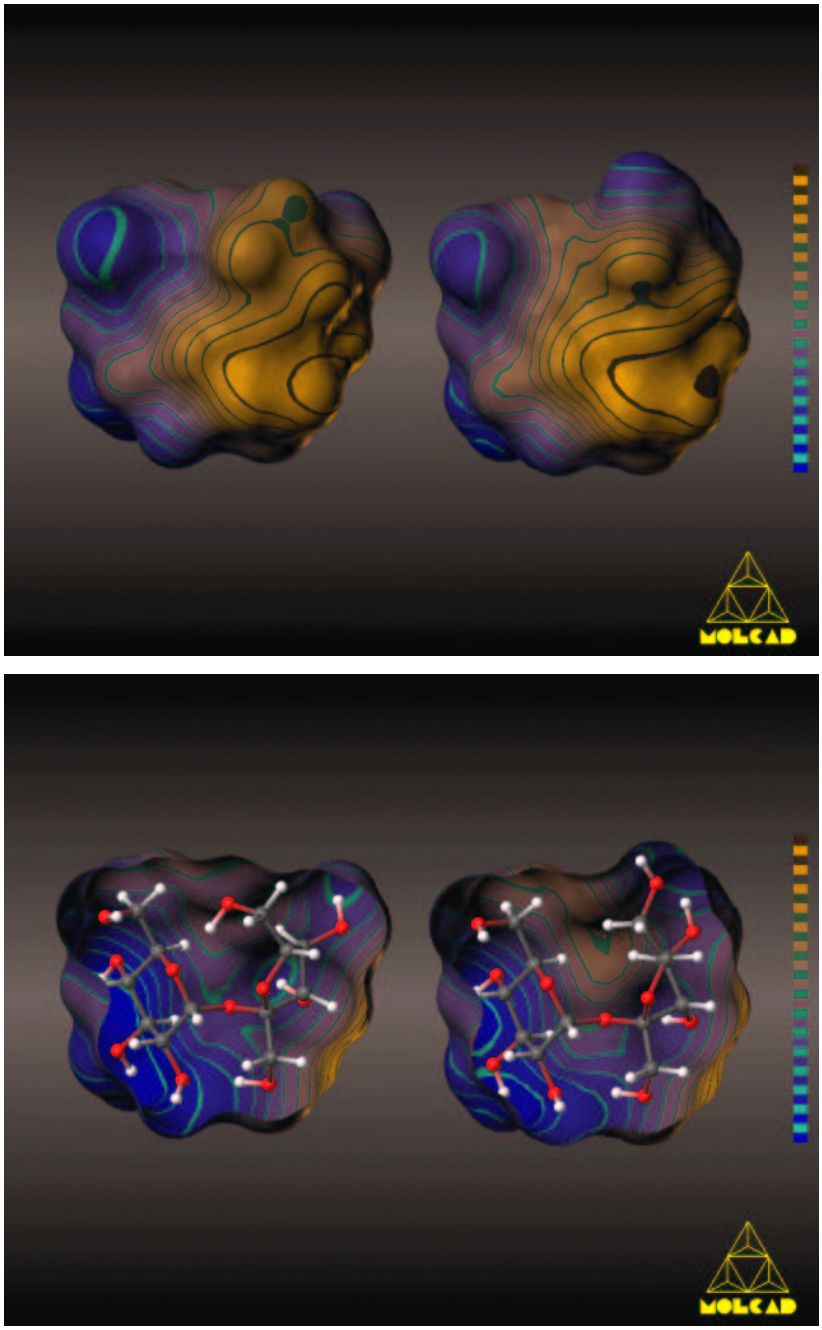


Fig. 2-6. Molecular lipophilicity pattern (MLP) of sucrose as distributed over the contact surface in 32 colors ranging from yellow-brown (most hydrophobic) to blue (most hydrophilic area): on the *left* side each, the MLP for the solid state conformation as derived from neutron diffraction data[25], on the *right* the MLP for the conformation likely to prevail in solution. Apparently, the hydrophobic and hydrophilic regions are located on opposite sides of the molecules.

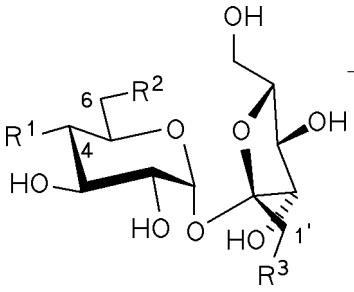
In keeping with these notions, the location of the tripartite AH-B-X glucophore emerges in the form indicated in Fig. 2-1: the glucosyl-2-OH and 3-OH being the proton donor (AH) and proton acceptor (B) parts engaging in simultaneous hydrogen-bonding to a complementary (inverse) AH-B system on the receptor protein, whilst the hydrophobic X-part is a region centered around H-3 of fructose.

The Tripartite AH-B-X Glucophore in Sucrose Derivatives

In securing corroborative evidence for the location of the AH-B-X glucophore, the sucrose derivatives considered are limited (for elimination of steric misfits) to those modified either by inversion, deoxygenation, and *O*-methylation of individual hydroxyl groups, or by their replacement with halogens.

The most direct method of probing into the location of the AH-B-X glucophore appears to be the replacement of a given hydroxyl group in sucrose by hydrogen and assessment of its effect on sweetness. As of now, however, sweetness data are available only for three of the eight mono-deoxy-sucroses, i.e. the 4-deoxy (**4**), 6-deoxy (**5**) and 1'-deoxy derivatives (**6**) (Table 2-1). All are less sweet than sucrose[60,61], yet the fact that sweetness is not lost altogether may be taken as an indication that none of the hydroxyl groups removed (i.e. the 4-OH and 6-OH of glucose, and 1'-OH of fructose) occupy positions detrimental for eliciting the sweet response. Accordingly, the existence of the intramolecular 2-O^{g} ··· HO-1^{f} hydrogen bond in sucrose is obviously no prerequisite for proper interaction with the sweet receptor, a notion that is similarly emerging from the sweetness of 1'-*O*-methyl- and 1'-chloro-1'-deoxy derivatives of sucrose (cf. below). Unfortunately, sweetness data on the other five deoxy-sucroses (2- and 3-deoxy in particular) or any of the 28 possible dideoxy-sucroses, which would further contribute to this issue, are not yet available.

Table 2-1. Sweetness of 4-deoxy- (**4**), 6-deoxy- (**5**), and 1'-deoxy-sucrose (**6**).

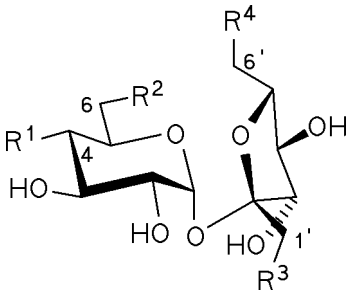


	R^1	R^2	R^3	relative sweetness*	refs.
sucrose	OH	OH	OH	SS	–
4	H	OH	OH	S	60
5	OH	H	OH	S	60
6	OH	OH	H	S	60-62

* S = sweet, SS = very sweet

Next to the deoxygenation, *O*-methylation of individual OH-groups in sucrose appears to be a suitable means of probing binding sites, provided that the steric expansion introduced by the OH → OMe conversion does not detract from the taste assessment. Such an impairment appears to be small, if at all, as evidenced by the data available for four mono- (**7** – **10**) and four dimethyl ethers of sucrose (**11** – **14**, Table 2-2)[60-63]: any of the three primary OH-groups in sucrose may be *O*-methylated without losing sweetness, which also applies to the glucose-4-OH. In all of these methyl ethers the glucose-2- and 3-OH groups, i.e. the structural AH-B requirements of Fig. 2-1, remain untouched, thus their sweetness data may be taken as an affirmation of the glucophore assignment. Curiously, no sweetness data of sucrose derivatives methylated at O-2, O-3, or at both of these positions, which would shed further light on this issue, are available.

Table 2-2. Sweetness of methyl ethers of sucrose.

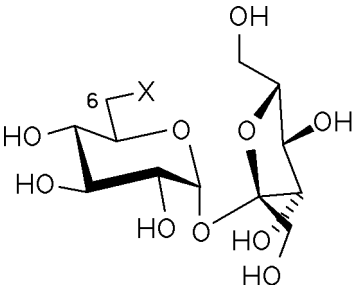


	R1	R2	R3	R4	relative sweetness*	refs.
sucrose	OH	OH	OH	OH	SS	–
7	OMe	OH	OH	OH	S	63
8	OH	OMe	OH	OH	SS	60
9	OH	OH	OMe	OH	SS	61,62
10	OH	OH	OH	OMe	SS	63
11	OMe	OMe	OH	OH	S	63
12	OMe	OH	OH	OMe	S	63
13	OH	OMe	OH	OMe	SS	63
14	OH	OH	OMe	OMe	S	63

* S = sweet, SS = very sweet

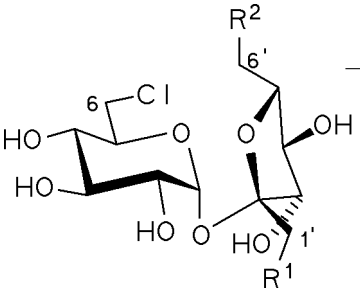
The 6-position in the glucose portion of sucrose appears to be a sensitive one with respect to the steric bulk introduced by its chemical modification. Whilst the 6-deoxy (**5**) and 6-*O*-methyl (**8**) derivatives are as sweet as sucrose, the 6-*O*-acetate (**15**) is only slightly sweet, the 6-benzyl ether (**16**), the 6-benzoate (**17**), and 6-phosphate (**18**) are bitter, as is the 6-chloro-6-deoxy compound (**19**, cf. Table 2-3). In the latter case, the obvious misfit introduced by the 6-chlorine substituent may be overcome by increasing the hydrophobicity of the fructose portion: 6'-chlorination (→ **20**) removes the bitterness, and chlorination at both primary positions of the fructose portion result in a molecule (**21**) with enhanced sweetness (cf. Table 2-4)[21,62,64].

Table 2-3. Taste characteristics of sucrose derivatives modified at the glucose C-6.



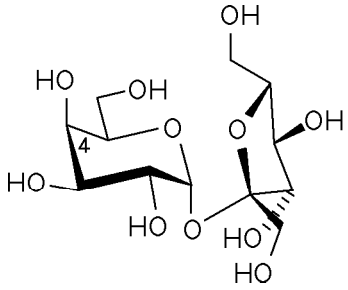
	X	taste	refs.
sucrose	OH	sweet	–
5	H	sweet	60,62
8	OMe	sweet	60,62
15	OAc	slightly sweet	60,62
16	OBn	bitter	60,62
17	OBz	bitter	60,62
18	OPO_3H	bitter	60,62
19	Cl	bitter	60,62,64

Table 2-4. Taste characteristics of 6-chloro-6-deoxy-sucroses.

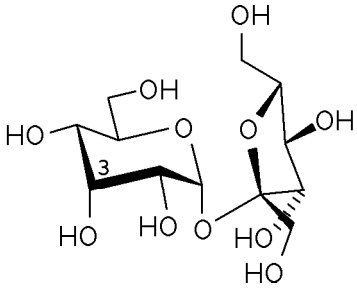


	R^1	R^2	taste (sucrose sweetness = 1)	refs.
19	OH	OH	bitter	21,62,64
20	OH	Cl	not sweet	64
21	Cl	Cl	25	21,62

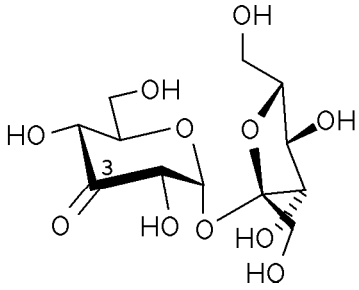
Configurational changes in the glucose portion of sucrose seem to have a more pronounced effect on the sweetness: its 4-epimer, the "*galacto*-sucrose" (**22**) has very low sweetness[63], and the 3-epimeric analogue "*allo*-sucrose" (**23**) is tasteless[65]. This clearly indicates subtle stereochemical requirements for the substrate on entering and / or being embedded into the receptor site(s). In the case of the 3-epimer **23** this may be rationalized via the change of the steric requirements of the AH-B site, an axial 3-OH being incapable of functioning as the hydrogen bond accepting B component.



galacto-sucrose
22



allo-sucrose
23



3-*keto*-sucrose
24

That 3-*keto*-sucrose (**24**) is sweet[65] goes along well with the AH-B-X tripartite glucophore assignment of Fig. 2-1, inasmuch as the 3-carbonyl function retains the hydrogen bond acceptor capabilities.

Less well comprehensible is, at first sight, the very low sweetness of *galacto*-sucrose (**22**)[63], in which the 2-OH and 3-OH of the hexosyl portion, i.e. the AH-B functionalities suggested in Fig. 2-1, are intact. A clue as to the possible reasons emerges from a comparison of the conformations of sucrose in solution, which features a *gg* arrangement[66] of the glucosyl-6-CH_2OH relative to the pyranoid ring (Fig. 2-7 **A**), with the respective arrangements for its 4-epimer **22**. The PIMM-program generated, minimum energy conformations for **22** result in the forms depicted in Fig. 2-7, **B**–**D**, which only differ by the orientation of the galactosyl-6-CH_2OH. Thereby, the *gg* form **B**, due to formation of a stabilizing intramolecular hydrogen bond 6-OH^g ··· O-4^g, comes out to be about 4 and 8 kJ/mol more stable than the *tg* (**C**) and *gt* rotamers (**D**), yet it is clear that this only prevails *in vacuo*. For solution, there is abundant evidence in the literature[53,67-73] that the sterically unfavorable 1,3-*syn*-interactions between 4- and 6-hydroxyl groups of a hexopyranose are evaded by solvation, hence, rotamers **C** and **D** of Fig. 2-7 will have to be entered into structure-sweetness considerations. Correspondingly, the sucrose conformation of Fig. 2-7 **A** is to be set against those of *galacto*-sucrose depicted in Fig. 2-7 (**C**, **D**): inversion of configuration at C-4 of sucrose entails a distinctive change in the rotameric preference at the 6-OH, which is an *a priori* sterically sensitive position (*vide supra*, Table 2-3). Thus, the very low sweetness of *galacto*-sucrose may be taken as an indication that the C-6 substituent in the aldose portion of sucrose is not only sensitive towards steric bulk – any significant increase resulting in the loss of sweetness (cf. Table 2-3) – but also in its orientation to the pyranoid ring, with a *gg* arrangement conceivably being favored.

Another factor responsible for the substantial decrease in sweetness on inversion of the sucrose-4-OH may be found in the shift of the molecular lipophilicity pattern (Fig. 2-8), of which the hydrophilic (blue) area centered around C-3 of the glucose unit is shifted to the upper side as compared to sucrose in Fig. 2-4. That, on the other hand, 4-chloro-4-deoxy-*galacto*-sucrose exhibits a 5-fold higher sweetness than sucrose[64] can be attributed to the hydrophobic substituent at C-4 that changes the overall shape of the molecule and its MLP substantially (as observed, for example, for the pyranoid portion of sucralose, cf. Fig. 2-9 – 2-11 below).

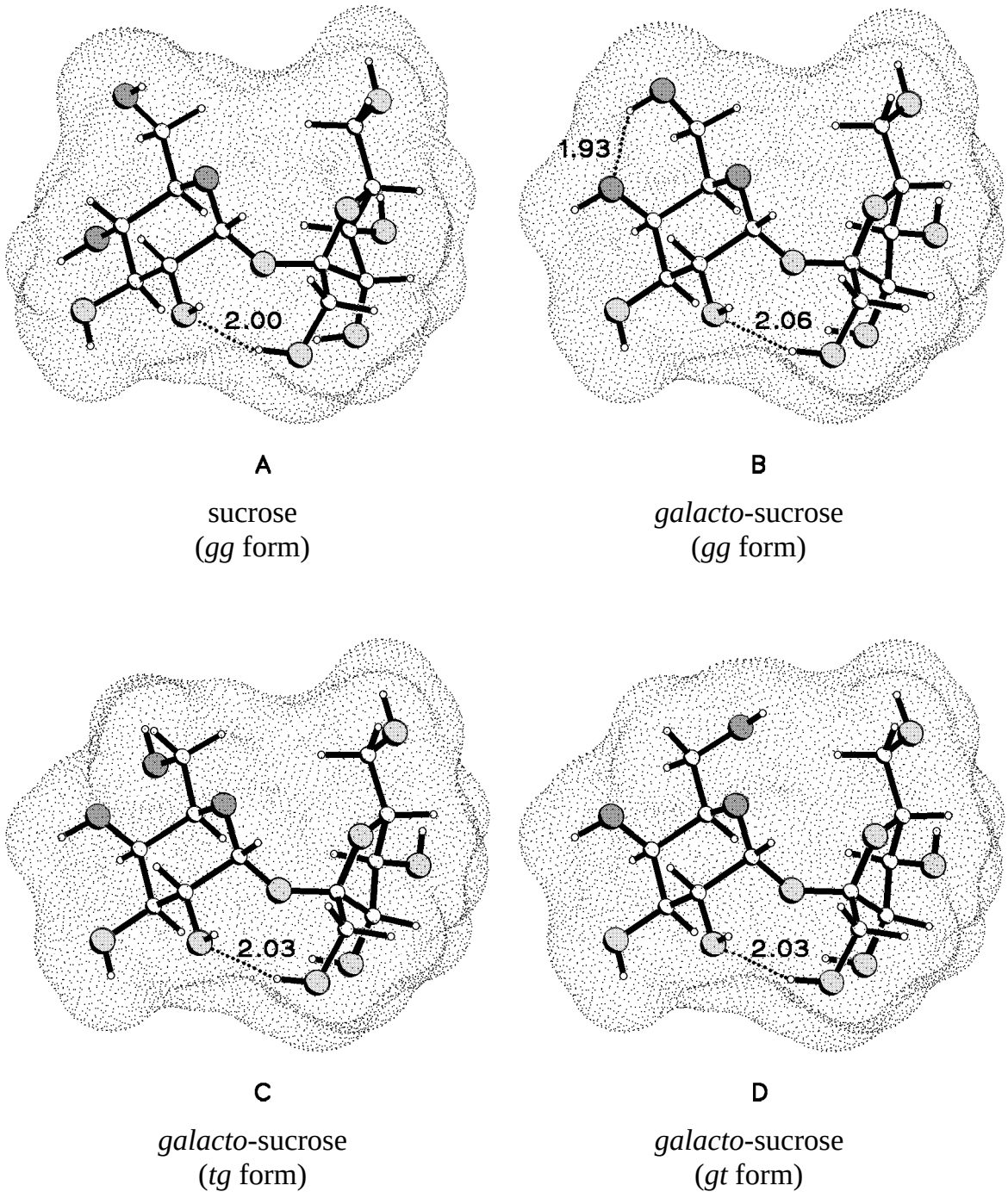


Fig. 2-7. Contact surface (in dotted form) of sucrose (**A**, featuring a *gauche-gauche* (*gg*) arrangement[66] for the glucosyl-6-OH relative to the pyranoid ring) as compared to the three conformers of *galacto*-sucrose (**22**) generated by PIMM force field calculations: the *gg* form **B** (in vacuum only), stabilized by an intramolecular hydrogen bond 6-OH^{g} ··· O-4^{g}, and the two forms relevant in solution, i.e. the *trans-gauche* (*tg*, **C**) and the *gauche-trans* (*gt*, **D**) rotamers. The oxygens involved in the designations and discussion (cf. text) are accentuated by filling.

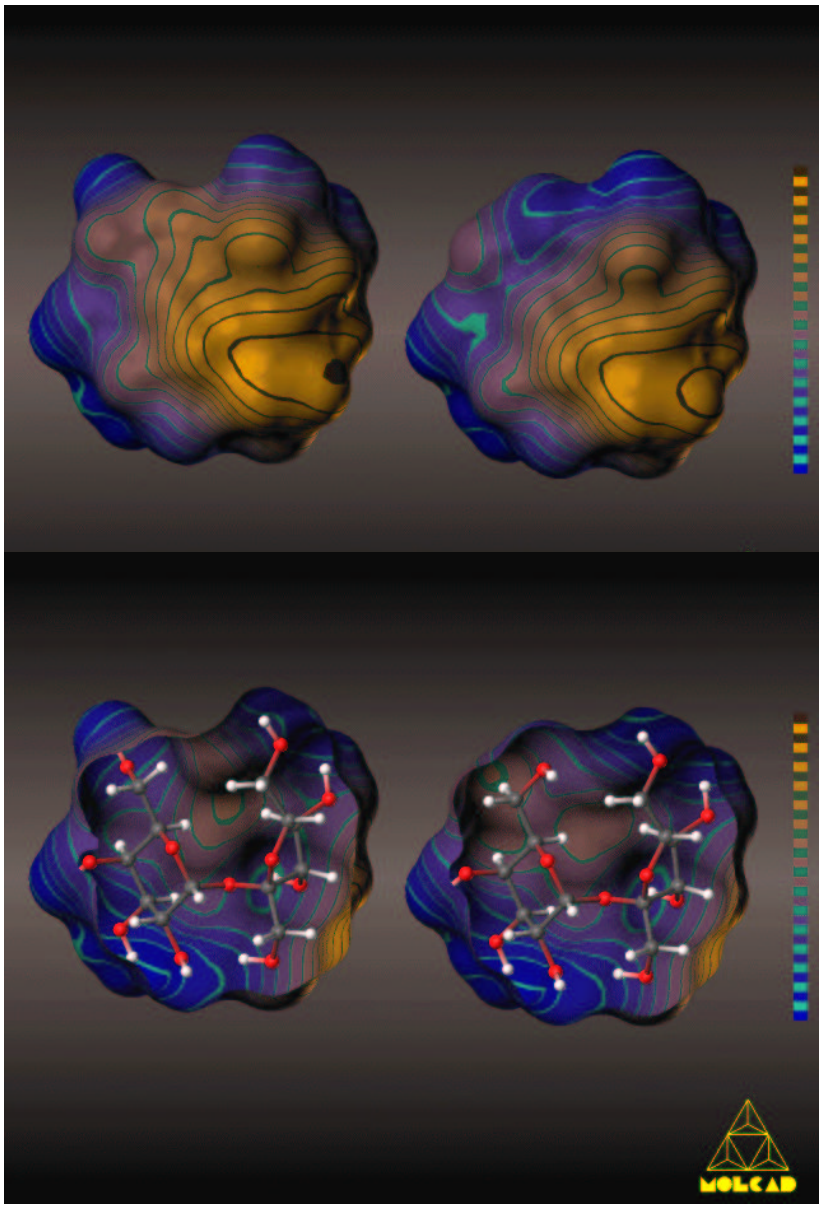


Fig. 2-8. Hydrophobicity pattern (MLP) of the *tg* (*left*) and *gt* rotamers (*right*) of *galacto*-sucrose (**22**), corresponding to the forms **C** and **D** in Fig. 2-7.

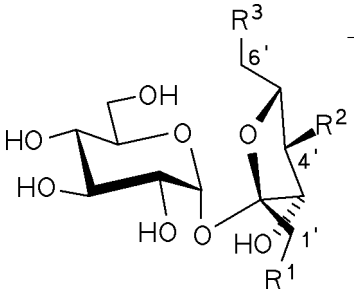
Deoxy-Halo-Sucroses

Unlike the deoxy- and *O*-methyl-sucroses discussed so far, whose sweetness is of about the same or lower intensity than that of the parent sugar, some deoxy-halo derivatives of sucrose exhibit substantially enhanced sweetness, in specific instances even several thousand times that of sucrose. Following the discovery of the first compound of this type by Hough and Phadnis in 1976[74], an impressively large number of deoxy-halo-sucroses has been synthesized and evaluated for their potency in sweet taste perception[21-23,62,64,74-85]. The altogether 35 compounds listed in the

following Tables provide a large body of experimental evidence that should be rationalizable in terms of the AH-B-X conceptual assignments made in Fig. 2-1.

The AH-B-X assignment of Fig. 2-1 implies that the hydrophobic cleft of the taste receptor protein corresponds to the hydrophobic region in the fructose moiety of sucrose (cf. Fig. 2-6). In keeping with this notion, it may be predicted that an increase of hydrophobicity in the fructose portion – i.e. along the yellow-brown portion in the MLP (Fig. 2-6) – favors binding to the corresponding hydrophobic site in the receptor, and hence enhances sweetness. Indeed, the data collected for the compounds **25 – 30** (Table 2-5) support this rationalization, since replacement of the fructose hydroxyl groups at the 1'-, 4'-, and / or 6'-position by chlorine or bromine uniformly leads to compounds sweeter than sucrose.

Table 2-5. Relative sweetness of deoxy-halo-sucroses modified in the fructose portion.



	R^1	R^2	R^3	relative sweetness	refs.
sucrose	OH	OH	OH	1	–
25	Cl	OH	OH	20	21,62,64,80
26	OH	OH	Cl	20	21,62,64
27	Cl	Cl	OH	30*	81
28	Cl	OH	Cl	80	21,62
29	Br	OH	Br	80	84
30	Cl	Cl	Cl	100	81

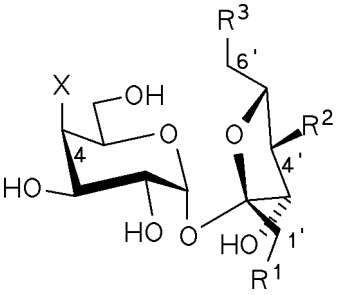
* In Table XIX on p. 266 of Lee's review[4] the relative sweetness for **27** is erroneously listed as being 3500.

Analysis of the sweetness characteristics of the numerous 4-halo-4-deoxy-*galacto*-sucroses (Table 2-6) in terms of the AH-B-X assignment of Fig. 2-1 is particularly informative. A shift of the substantially hydrophilic glucosyl-4-OH of sucrose (blue area in Fig. 2-6) from the equatorial to the axial orientation (→ *galacto*-sucrose (**22**), cf. Fig. 2-8), together with the accompanying change in the rotameric arrangement of the 6-OH^g (cf. Fig. 2-7) results in a near loss of sweetness[63]. However, when placing a pronouncedly hydrophobic substituent such as chlorine into the very same axial position, the sweetness, relative to sucrose, is enhanced by a factor of 5 (compound **31** in Table 2-6).

The comparison of the sweetness of 4-chloro-4-deoxy-*galacto*-sucrose (**31**) with that of its analogs **32 – 41** is particularly instructive, inasmuch as the successive replacement of the fructose 1'-, 4'-, and 6'-OH groups translates into substantially

increased sweetness values, the 1',4',6'-tribromo analog **41** featuring a 1400 fold enhancement over **31**. An essentially identical trend is observed in the 4-deoxy-4-fluoro-*galacto*-sucroses **42** – **44** (a 10 fold increase of sweetness from the 4,6'-difluoro **42** to the 4,1',6'-trifluoro compound **43**), and very markedly, in the 4-bromo analogs **45** – **47**, in which the enhancement of sweetness perception reaches a solitary maximum.

Table 2-6. Relative sweetness (sucrose = 1) of 4-deoxy-4-halo-*galacto*-sucroses.



	Glucose	– Fructose –			relative	
	X	R^1	R^2	R^3	sweetness[a)]	refs.
sucrose	OH_{eq}	OH	OH	OH	1	–
31	Cl	OH	OH	OH	5	21,64,80
32	Cl	OH	OH	Cl	50	84
33	Cl	Cl	OH	OH	120	21,62
34	Cl	OH	Cl	Cl	160	81
35	Cl	Cl	Cl	OH	220	81
3[b)]	Cl	Cl	OH	Cl	650	78
36	Cl	Br	OH	Br	800	84
37	Cl	Cl	F	Cl	1000	81
38	Cl	Cl	Cl	Cl	2200	79,82
39	Cl	Cl	Br	Cl	3000	81
40	Cl	Cl	I	Cl	3500	78,81
41	Cl	Br	Br	Br	7000	81
42	F	OH	OH	F	4	84
43	F	F	OH	F	40	78
44	F	Cl	Cl	Cl	200	81
45	Br	Cl	OH	Cl	375	84
46	Br	Br	OH	Br	800	84
47	Br	Br	Br	Br	7500	78,81
48	I	I	OH	I	120	78,84

a) All of these data were retrieved from the original literature as indicated by the respective references. It should be noted that a number of sweetness values given in Lee's review (*Adv. Carbohydr. Chem. Biochem.* **1987**, *45*, Table XIX on p. 266) are at error, most notably the data listed for compound **40** (7000 instead of 3500), and **41** (30 instead of 7000). – b) Sucralose.

Of these compounds in Table 2-6, sucralose (**3**), a non-caloric high-potency sweetener recently approved for food use[86], was selected to further probe into the hydrophobic / hydrophilic portions of the molecule by computer modeling, an intent facilitated by the availability of its X-ray structure[87]. In Fig. 2-9 the molecular geometries and their contact surfaces are depicted for two forms of sucralose, **A**

representing the crystalline state conformation based on X-ray data, **B** the state likely to prevail in solution[88], as generated by force field optimizations. It is noteworthy that the directionality of the intersaccharidic hydrogen bond is reversed on going from the crystal (**A**, 2-OH^{g} ··· O-3^{f}) to the lowest energy, computer simulated form (**B**, 2-O^{g} ··· HO-3^{f}).

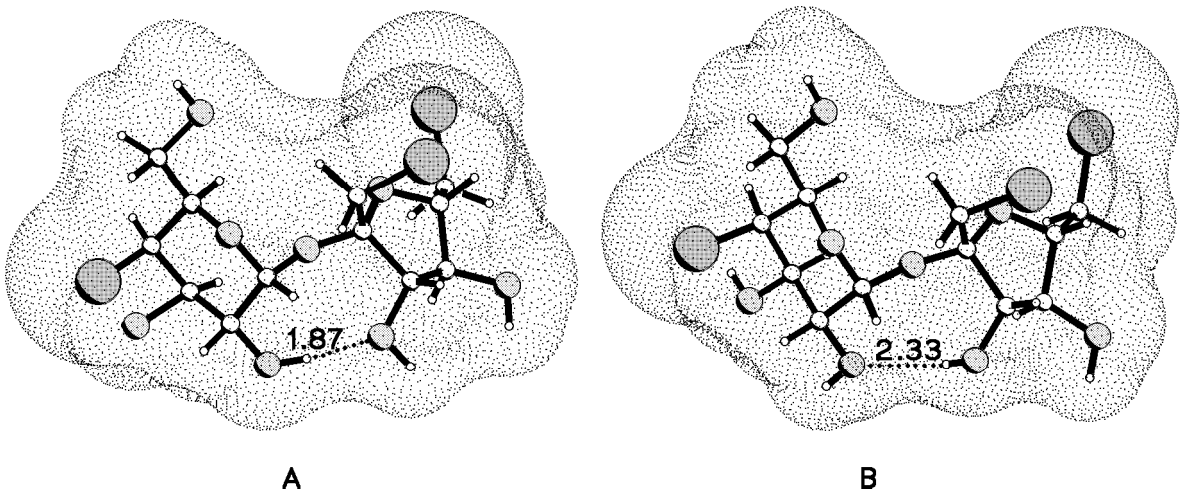


Fig. 2-9. Molecular geometry and dotted contact surface of sucralose®, the large balls representing the three chlorine atoms at positions 4 of the hexose, and positions 1 and 6 of fructose. (**A**): solid state conformation[87], (**B**): lowest energy conformer emerging from PIMM88 force field calculations. Both models differ mainly in the directionality of the interresidue hydrogen bond 2-O^{g} ··· O-3^{f}, the *right* model corresponds closer to the conformation adopted in solution[88].

Portraying on this contact surface the MOLCAD-generated MEP-pattern, it is evident from Fig. 2-10 that the form relevant for solution (closed and opened form on the right each), is as pronouncedly electropositive (red) as observed for sucrose (cf. Fig. 2-5), obviously due to an analogous direction of the 2-O^{g} ··· HO-3^{f} hydrogen bond.

The hydrophobicity pattern pictured in Fig. 2-11, expectedly shows the two chlorine atoms in the fructose portion to be the hydrophobic center (X-site), now being extended over the entire "outside" region of fructose (as compared to sucrose, cf. Fig. 2-6). Another obvious similarity with sucrose is the fact that hydrophobic (yellow-brown) and hydrophilic (blue) regions are located on opposite sides of the molecule, seemingly little disturbed by the third chlorine at C-4 of the pyranoid ring, which – as evident from Fig. 2-11 – is less hydrophobic than the other two.

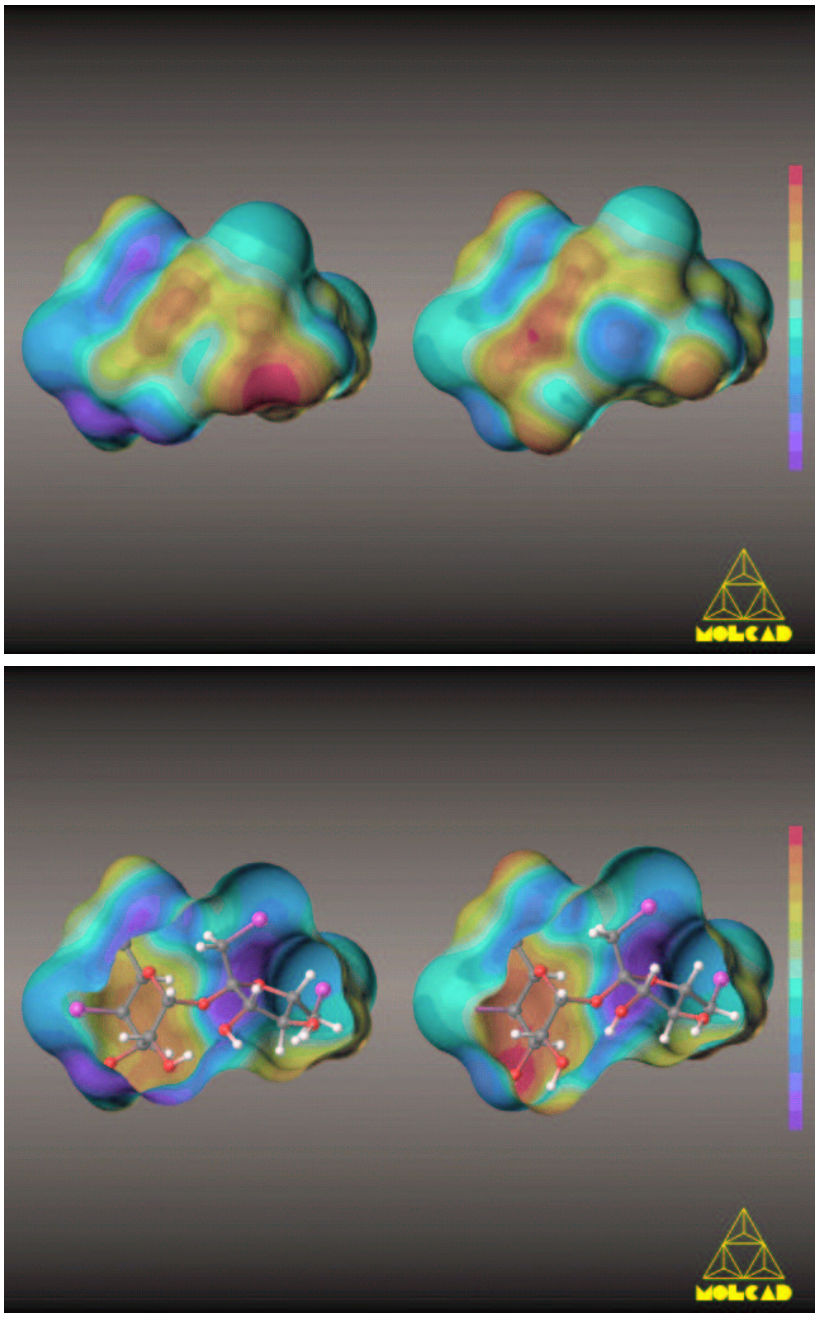


Fig. 2-10. Molecular electrostatic potential (MEP) profile on the contact surface of sucralose in the crystal[87] (*left*) and in the form generated by calculations (*right*), which conceivably has closer correspondence to the form prevalent in solution (red: electropositive, violet: electronegative).

An interesting relationship between sweetness and the nature of the halo substituent in sucralose analogs is revealed by the data listed in Table 2-7. Sucralose (4,1',6'-trichloro-trideoxy-*galacto*-sucrose, **3**), is 16 fold sweeter than its trifluoro analog **43**, and approximately 5 times sweeter than the triiodo compound **48**,

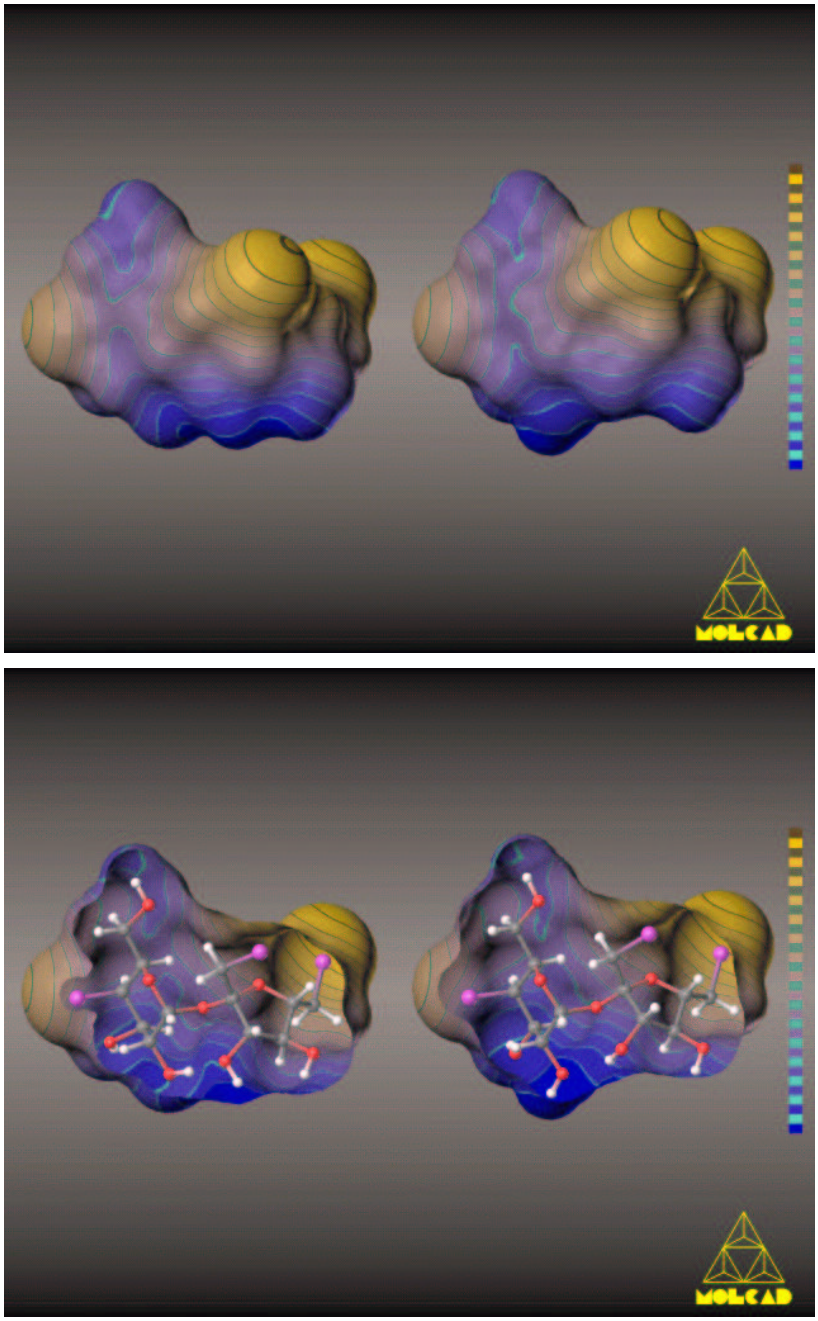
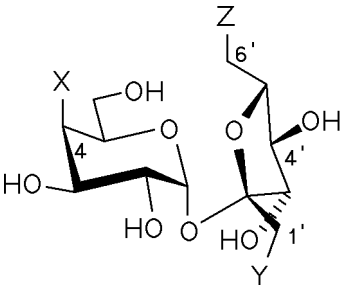


Fig. 2-11. Molecular lipophilicity patterns (MLP's) of sucralose in the solid state structure[87] (*left*), and the computer-simulated conformation (*right*). The reversal of the direction of the interresidue hydrogen bond from 2-OH^{g} ··· O-3^{f} (*left*) to 2-O^{g} ··· HO-3^{f} (*right*) results in a concentration of the hydrophilic area (blue) around O-2^{g}. The two chlorine atoms of the fructose unit are located within the most hydrophobic (yellow-brown) region.

indicating that fluorine is too small, and iodine is too big to properly fit into the receptor binding site(s). The best fit appears to be provided by bromo substituents in the fructose 1'- and 6'-positions, as evidenced by the compounds **36** and **46**, which are 800 times sweeter than sucrose[78,84].

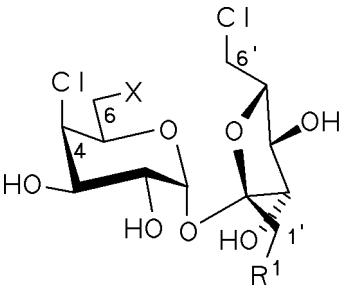
Table 2-7. Relative sweetness (sucrose = 1) of sucralose (**3**) analogs.



	X	Y	Z	relative sweetness	refs.
43	F	F	F	40	78
3*	Cl	Cl	Cl	650	78
36	Cl	Br	Br	800	84
45	Br	Cl	Cl	375	84
46	Br	Br	Br	800	84
48	I	I	I	120	78,84

* Sucralose

Table 2-8. Relative sweetness (sucrose = 1) of some 4,6'-dichloro substituted *galacto*-sucroses (analogs of sucralose **3**) modified at C-6.



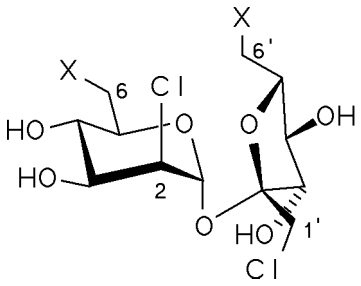
	X	R^1	relative sweetness	refs.
3*	OH	Cl	650	78
49	Cl	OH	4	80
50	Cl	Cl	200	80
51	H	Cl	400	78,83
52	OMe	Cl	500	78,83
53	O*i*Pr	Cl	not sweet	78

* Sucralose

Another probe into the effect of the 6-substituent on sweetness is provided by the sucralose analogs additionally substituted at C-6 (Table 2-8): in comparison to compound **3**, the sweetness of the 4,6,6'-trichloro isomer **49**, which lacks the sweetness enhancing 1'-chloro substituent in the hydrophobic center of the fructose portion, drops dramatically (650 → 4), a decrease that is only partially made up by introducing that 1'-chloro group (4 → 200 for **50**), due to the presence of critical 6-Cl substituent (cf. Table 2-3). As expected from the discussion of 6-deoxy- and 6-*O*-methyl-sucrose (Table 2-1 and 2-2), modification of the 6-substituent by deoxygenation (→ compound **51**) and *O*-methylation (→ **52**) has only a minor effect on the intensity of the sweetness sensation. Yet, increase of the steric bulk at C-6 from OCH_3 (**52**) to $OCH(CH_3)_2$ (**53**) is seemingly fatal, as sweetness is lost altogether.

Oddly enough, sucrose derivatives modified at either the glucosyl-2-OH or 3-OH group, like the deoxy-, *O*-methyl- or chloro-analogs, are not available. Sweetness data on these have a major bearing on the AH-B assignment as in Fig. 2-1. The only

examples along these lines are the two 2-chloro-2-deoxy-mannosyl analogs. The dichloride **54** has been found to be "not sweet at all"[81,82], and the tetrachloride **55**, despite its additional hydrophobicity- and, hence, sweetness-enhancing 6'-chloro substituent, is "as bitter as quinine"[85].



54 X = OH (not sweet at all)[81,82]
55 X = Cl (very bitter)[85]

This finding, along with the observation that *allo*-sucrose (**23**) is not sweet, emphasizes that there are strict steric requirements for the arrangement of the 2- and 3-hydroxyl groups, and that their equatorial orientation is seemingly essential for eliciting the sweet sensation. This conclusion is also in accord with, but no absolute proof of the AH-B assignments to the 2- and 3-OH groups of the glucosyl moiety in sucrose.

By way of summary, the foregoing discussion of the sweetness of over 50 derivatives or analogs of sucrose provides ample evidence for placing the hydrophobic X-site of the Shallenberger-Kier glucophore onto the outside region of the fructose rather than to other parts of the sucrose molecule. This is borne out by the MLP's presented, and by the fact that an increase of the hydrophobicity in the fructose portion invariably results in an enhancement of sweetness. In keeping with the notion that the X part is mainly responsible for orientation of the molecule in entering and / or being embedded into the receptor site, it can be assumed, that this hydrophobicity-controlled "docking procedure" of the substrate into the hydrophobic cleft of the receptor is required for bringing the AH-B portions into the proper receptor positions to elicit the sweetness sensation via hydrogen bonding.

Placement of the hydrophobic X part into the fructose portion of sucrose leaves little alternative for the AH-B part: 4-deoxy- and 6-deoxy-sucrose are sweet, albeit less than sucrose (Table 2-1), as are the respective 4-*O*- and 6-*O*-methyl ethers (Table 2-2) – findings that render these positions most unlikely as those essential for eliciting the sweet response. In contrast, the data discussed above point towards location of the AH-B unit in the diol grouping made up by the glucosyl-2-OH and 3-OH groups. Both are situated within the most hydrophilic region of sucrose (cf. Fig. 2-6), and in principle, each may be assigned the B (or AH) part. Here, the AH part was assigned to the glucosyl-2-OH, mainly for the reason that the MEP pattern of

Fig. 2-5 indicates this hydroxyl group to be the most electropositive, and hence, is conceivably better disposed for engaging its OH-proton as a donor in the substrate-receptor hydrogen bond.

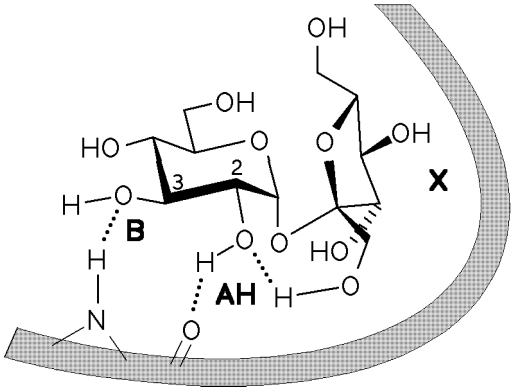


Fig. 2-12. Location of the tripartite AH-B-X glucophore ("sweetness triangle") in sucrose emerging from the computer-generated molecular electrostatic potential (MEP) profile and the molecular lipophilicity pattern (MLP).

In toto, the evidence for the assignment of the AH-B-X glucophore as depicted in Fig. 2-12, though not definitive, is a most useful working hypothesis for rationalizing sweetness data in sucrose derivatives, and surely worthy of further attention. It is expected that greater certainty will come from the sweetness data of the five remaining deoxy-sucroses (particular the 2- and 3-deoxy compounds), and of the four missing mono-methyl ethers, especially the 2-*O*-methyl and 3-*O*-methyl-sucroses. Other desirable derivatives with which the validity of the assignments could be further probed, are 2-*epi*-sucrose, i.e. its mannosyl analog, and its 3'-epimer, a *psico*-sucrose.

β-D-Fructopyranose: Conformations and Molecular Lipophilicity Profiles

D-Fructose crystallizes in the β-D-pyranoid form, as evidenced by solid state structural data[89,90]. 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[93-95] (cf. Fig. 2-13) 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 entirely, a conclusion that is supported by the parallelism of decrease of sweetness and of β-*p*-form (in the equilibrium tautomeric mixture) on increasing the temperature.

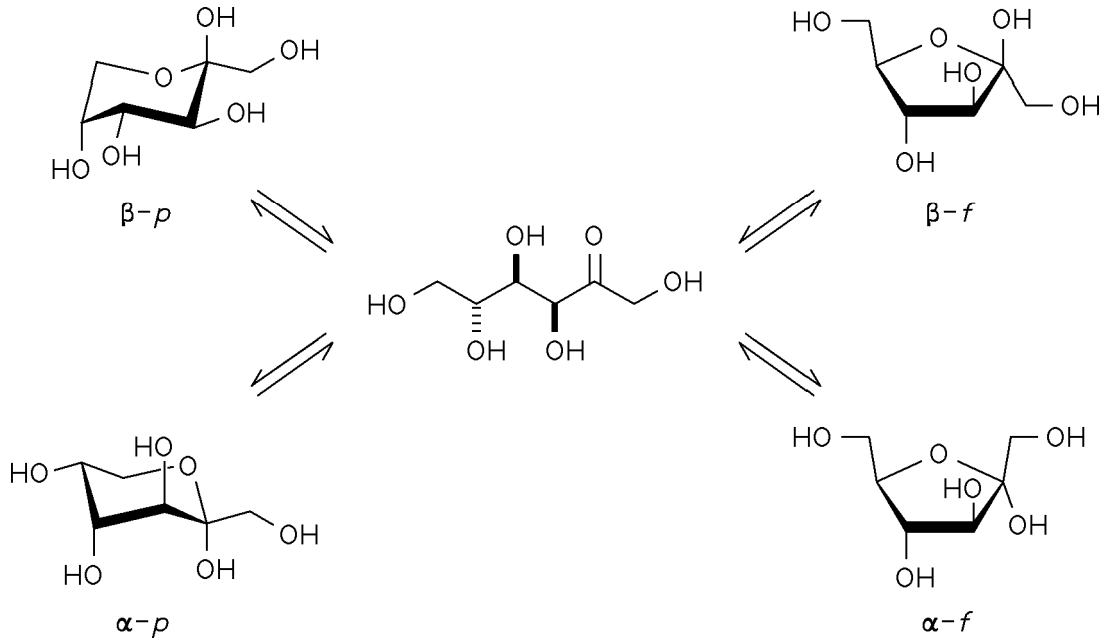


Fig. 2-13. Tautomeric forms of D-fructose (**2**). For an equilibrated aqueous solution at 25°C the composition is 73% β-*p*, 20% β-*f*, 5% α-*f*, and 2% α-*p* forms[94], the acyclic *keto*-form is negligible.

In consequence, fructose-sweetness considerations are all based on the β-*p* form, and several assignments for the tripartite AH-B-X glucophore have been advanced: (i) Shallenberger *et al.*[2,93,96], intuitively, and Lindley & Birch[97], on the basis of consideration of model compounds[97], arrived at the anomeric 2-hydroxyl group and the hydroxymethyl oxygen as the AH-B couple, respectively (Fig. 2-14, i). The inverse assignment (ii) was suggested by Szarek *et al.*[98-100] and by Mathlouthi & Portmann[20], considering calculations of the net atomic charges and the relative basicities of the hydroxyl groups[98], and IR-data rationalizations[20].

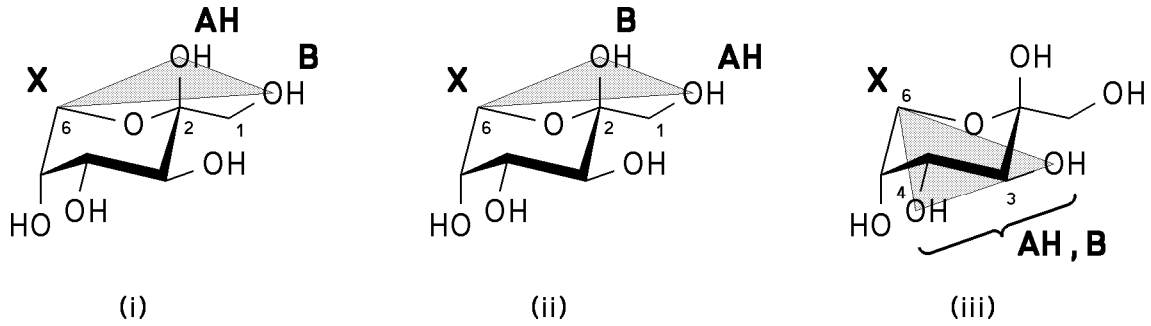


Fig. 2-14. Location of the tripartite AH-B-X glucophore in β-D-fructopyranose as suggested by Shallenberger[2,93,96] and Lindley & Birch[97] (i), by Szarek *et al.*[98-100] and Mathlouthi & Portmann[20] (ii), and by Birch *et al.*[101] (iii).

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 plays 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).

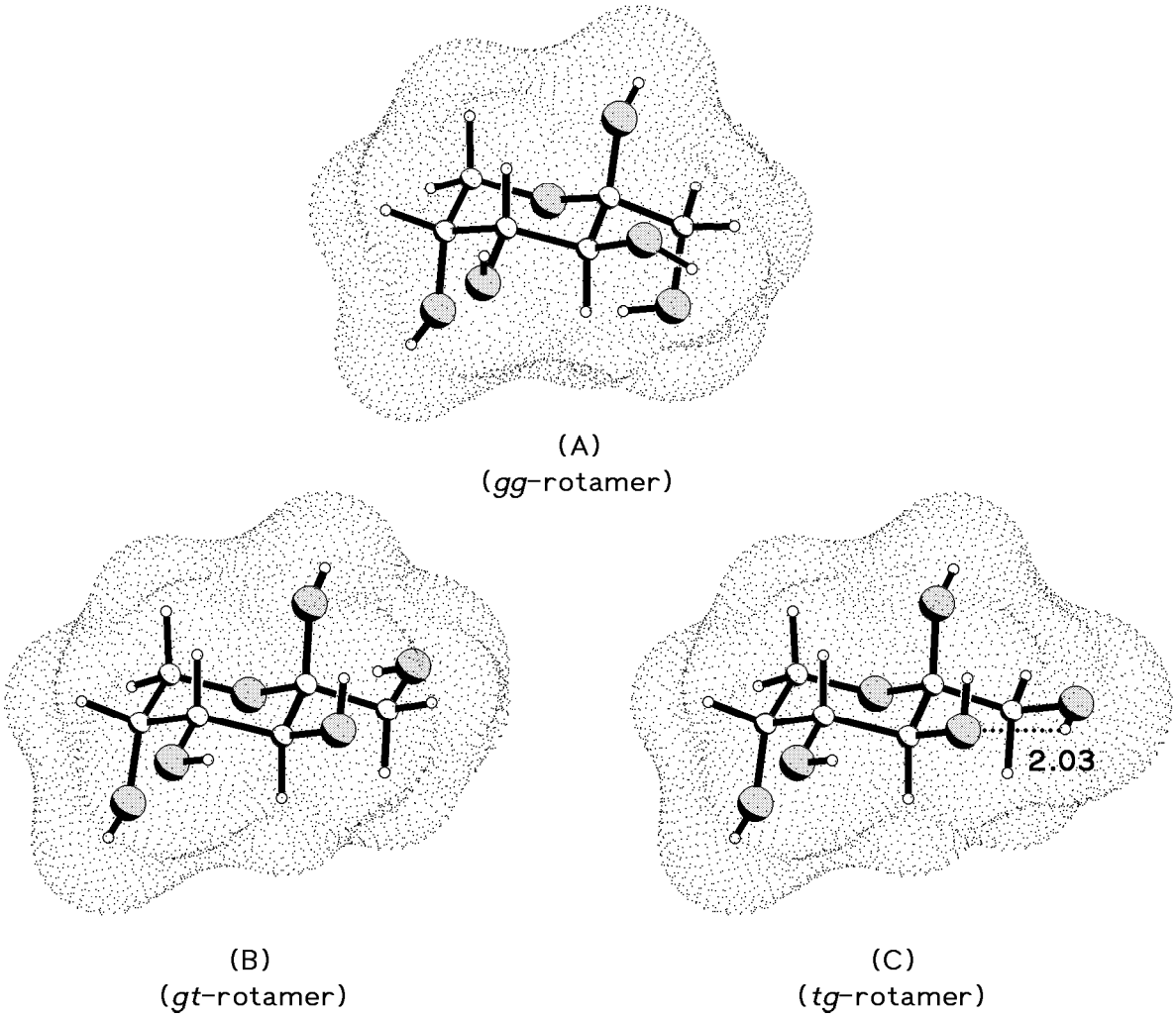


Fig. 2-15. The three staggered rotameric forms of the hydroxymethyl group in β-D-fructopyranose as derived from X-ray structural data[89,90] (**A**) and from force field calculations (**B** and **C**), and their respective contact surfaces (in dotted form). The *tg* rotamer (**C**), despite the unfavorable 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.

For generation of the MEP's and MLP's for β-D-fructopyranose, with which these assignments were to be probed, the relevant conformations of the hydroxymethyl group relative to the $^2C_5$-fixed pyranoid ring had to be determined. In the solid state, as evidenced by X-ray structural data[89,90], the *gauche-gauche* (*gg*) arrangement[102] of the primary hydroxyl group (**A** in Fig. 2-15) is realized. 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.

Semiempirical calculations of other conformations of **2** 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. This applies to the conformations emerging from very elaborate *ab initio* calculations[98,103] and AM1-based semiempirical investigations[99], as well as to those emanating from the more simple PIMM88 force field methodology. From the latter, the *tg* rotamer **C** (Fig. 2-15) comes out to be the global minimum energy conformation, despite the steric constraints of the 1,3-diaxial-like arrangement of the 1-OH and 3-OH groups, which obviously are overcome (*in vacuo*) by the stabilizing effect of the intramolecular hydrogen bond 1-OH ··· O-3 (2.03 Å). 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 as the molecular conformations preferred in solution, for which the contact surfaces (Fig. 2-15) and the MLP's were generated. As is evident from Fig. 2-16, both forms have their most hydrophilic surface area (blue) centered around the fructose-4-OH, whilst the hydrophobic (yellow-brown) part(s) are associated with either of the two methylene groups: in the *gg* rotamer (left entries in Fig. 2-16), 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 (right in Fig. 2-16), where the hydrophobic surface areas of the 1- and 6-CH_2 groups are separated.

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, and, as such, being reminiscent of the hydrophobicity pattern of sucrose (Fig. 2-6). Thus, 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.

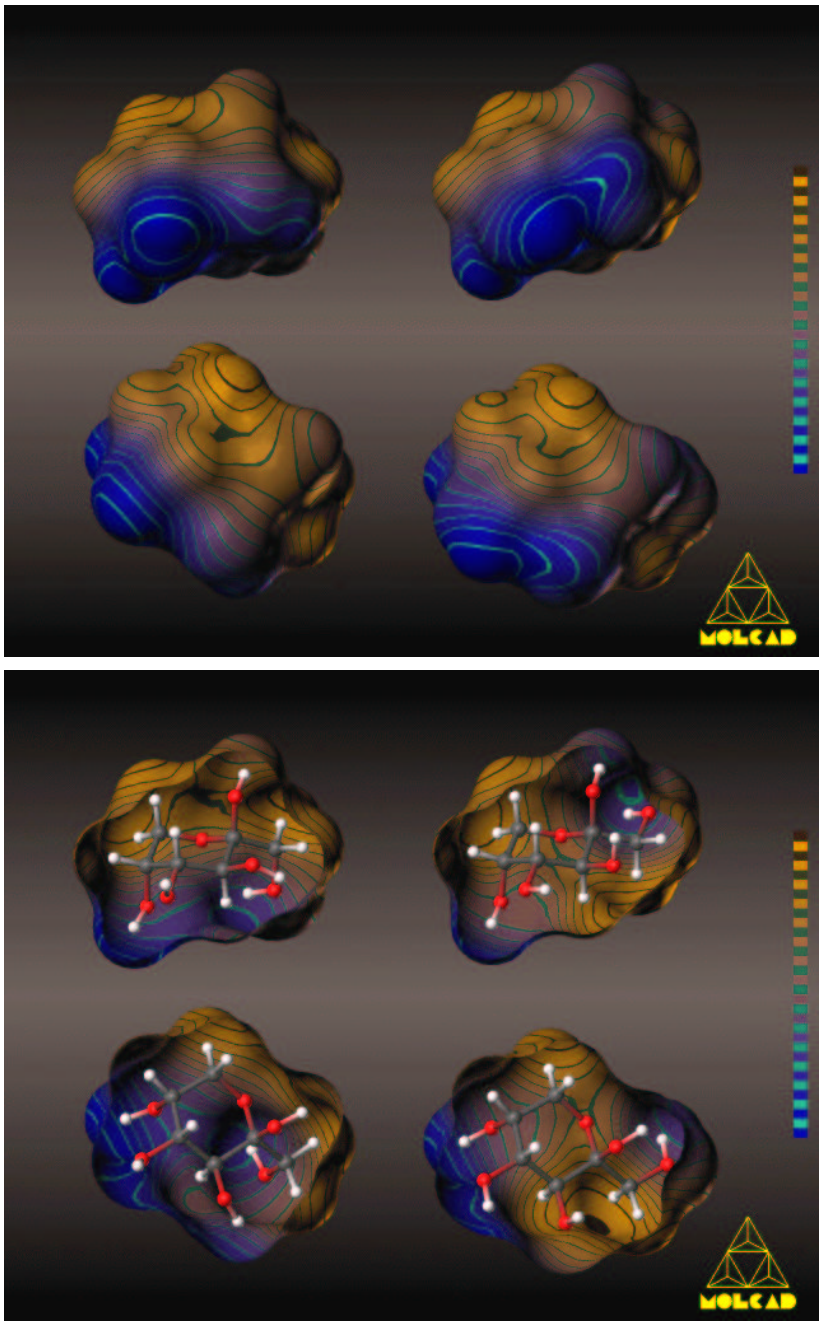


Fig. 2-16. Molecular lipophilicity patterns (MLP's) for two conformers of β-D-fructopyranose (**2**), differing in the disposition of the hydroxymethyl group relative to the pyranoid ring[102]: the *gg* conformer is depicted on the *left* side each, the *gt* form on the *right*. For each form, two representations were chosen, the *upper* corresponding in their orientations to those of Fig. 2-15 **B** and **C**, respectively. The modelings depicted underneath illustrate the opposite location of hydrophilic and hydrophobic regions.

Location of the AH-B entity on the basis of the MLP's of Fig. 2-16 – or the corresponding MEP patterns not depicted here – is seemingly difficult. Yet, the concentration of the most hydrophilic domains 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. The lipophilicity patterns 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. In this context, it is noteworthy that Szarek *et al.*[103] found as a result of *ab initio* investigations of **2** that O-4 in fructose exhibits enhanced basicity, while the secondary 4- and 3-OH protons seem to be relatively acidic, only being exceeded by the primary OH-group. These findings – in conjunction with the fact emerging from calculations of molecular electrostatic potentials[103] that "the O-4 atom would be predicted to be the most attractive site for protonation"[103] – may be taken as a hint for the importance of the 4-OH group in respect to structure-sweetness relationships.

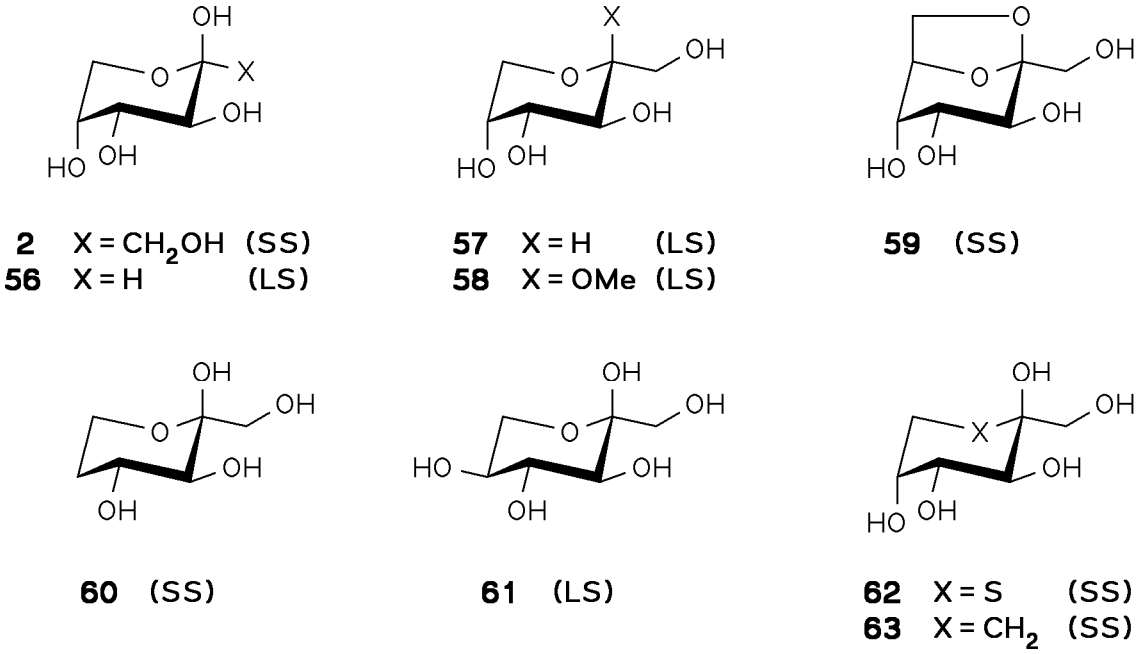


Fig. 2-17. Sweetness characteristics of analogs of β-D-fructopyranose (**2**). (SS: very sweet, LS: low sweetness)

Consideration of the few relevant fructose analogs, whose sweetness characteristics are known (Fig. 2-17), provides 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 (**56**), 2-deoxy-fructose (**57**, 1,5-anhydro-D-mannitol), and the 2-*O*-methyl derivative **58** (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 **59** is as sweet as fructose[104] attests to the contrary.

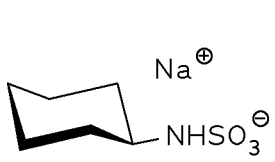
The sweetness characteristics of analogs **60** – **63** tally with either of the conjectural assignments in Fig. 2-14: the 5-hydroxyl group can be replaced by hydrogen (→ **60**) without loosing sweetness[105], and hence, as such is not an essential requirement for the sweetness sensation. However, its steric (axial) orientation is important since its configurational inversion to the 5-epimeric α-L-sorbo-pyranose (**61**) effects a substantial decrease in sweetness[106], possibly by introducing a steric misfit upon interaction with the receptor[105]. Similarly, the intense sweetness of the 6-thio (**62**)[107] and 6-carba analogs (**63**)[108] 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.

Although further evidence is required to settle this question unequivocally, as of now, major significance should be attributed on the MLP's obtained for the two fructose conformers likely to prevail in solution. These (Fig. 2-16) 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. Moreover, when focusing on the essentials contained in the lipophilicity patterns of the two fructose forms prevalent in solution (Fig. 2-16), the basic feature emerges that hydrophobic and hydrophilic regions are located on opposite sides of the molecule – a situation quite similar to the one observed for sucrose (Fig. 2-6). Thus, it may well be – and this receives fortification from the lipophilicity profiles of a number of non-carbohydrate sweeteners (see below) – that the opposite-side-distribution of hydrophobic and hydrophilic regions, the latter being capable for hydrogen bonding with the receptor, is the principal structural feature for eliciting the sweetness response, rather than an AH-B-X "sweetness triangle".

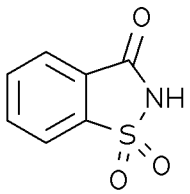
Molecular Lipophilicity Profiles of Non-Carbohydrate, High-Potency Sweeteners

The AH-B-X glucophore concept has not only been applied to sugars, but has been appreciated as the unifying criterion for such structurally diverse sweet substances as amino acids and a series of non-carbohydrate sweeteners such as cyclamate (**64**), saccharin (**65**), acesulfame (**66**), and aspartame (**67**)[3]. Serious reservations, however, must be advanced in regard to its general applicability, since it is known, for example, that the biologically active species of saccharin (**65**) and acesulfame (**66**) are the respective anions[109], in which it is difficult to locate the AH entity. Furthermore, the AH-B-X concept assumes that all sweet molecules interact with the same receptor in the same, or in an at least very similar way – an assumption which is quite questionable. Recent evidence[7,8] either points to several types of sweet receptors, or

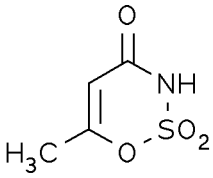
to different kinds of activations within the same one, if indeed sugars and high-potency sweeteners really elicit the sweet response via the same taste receptors. Despite these reservations it was obviously of interest to extend the molecular modeling techniques used above, to some representative non-carbohydrate sweeteners, as, for example, to compounds **64** – **68**.



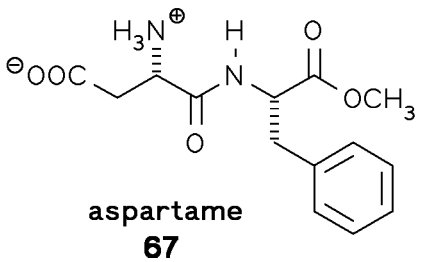
cyclamate
64



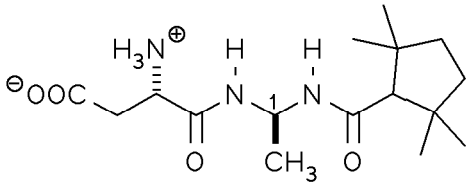
saccharin
65



acesulfame
66



aspartame
67



68

For this purpose, the solid state conformations were retrieved from the X-ray structural data available for saccharin (**65**)[110], acesulfame (**66**)[111], aspartame (**67**)[112], and the retro-inverso sweetener (**68**)[113] and used to calculate the respective contact surfaces (Fig. 2-18 and 2-20). In the case of cyclamate (**64**), for which an X-ray structure is lacking, the conformation was generated by PIMM calculations.

As clearly apparent from the contact surfaces of the three sulfamido sweeteners (Fig. 2-18), their overall molecular shapes are different, although the SO_2NH-element is placed on the left side of each compound in Fig. 2-18 to accentuate their common structural as well as three-dimensional feature, undoubtedly involved in eliciting the sweet response. However, when comparing their MLP's in Fig. 2-19, the similarity of distribution of hydrophobic and hydrophilic regions is amazing: that the sulfamido portion is the hydrophilic part of the molecule was to be expected. That the differences between a cyclohexyl ring (in cyclamate), an aromatic moiety (as in saccharin) and an acetoacetyl residue fixed in the enol form (as in acesulfame) level off to yield hydrophobic areas closely resembling each other – in the case of **65** and **66**, the two lower entries in Fig. 2-19, they are essentially identical – is most remarkable.

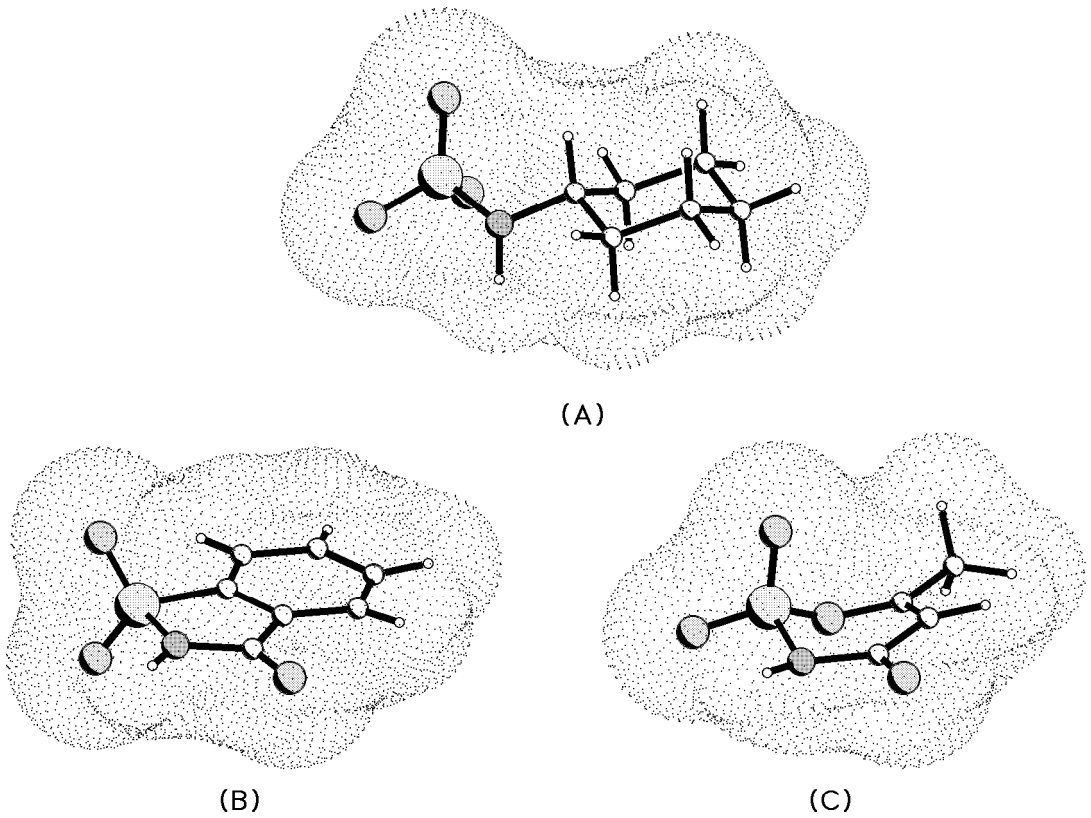


Fig. 2-18. Contact surface of cyclamate (**64**, **A**), saccharin (**65**, **B**), and acesulfame (**66**, **C**) in dotted form with a ball and stick model insert. The conformation of **64** (**A**) was generated by force field calculations, those of **65** (**B**) and **66** (**C**) were modeled according to the X-ray structural data of the corresponding sodium or potassium salts[110,111].

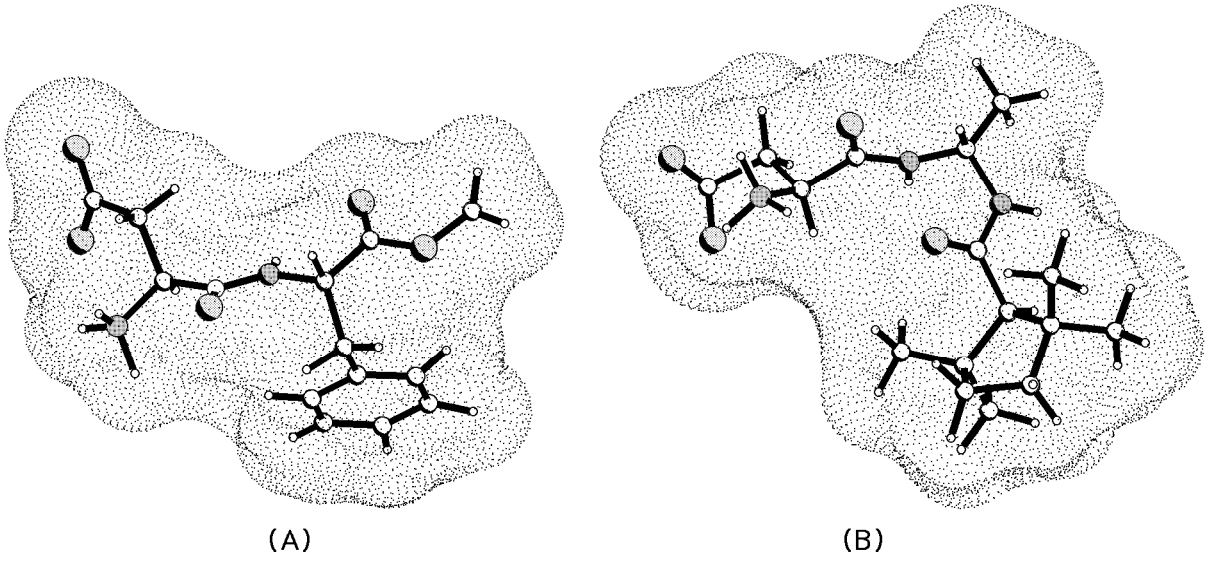


Fig. 2-20. Solid state conformation and contact surfaces, based on X-ray structural data[112,113] for **A**: the commercial dipeptide sweetener aspartame (**67**, "Nutrasweet"®), and **B**: the intensely sweet *N*-L-aspartyl-*N'*-(2,2,5,5-tetramethylcyclopentanyl)-carbonyl-(*R*)-1,1-diaminomethane (**68**), a retro-inverso dipeptide.

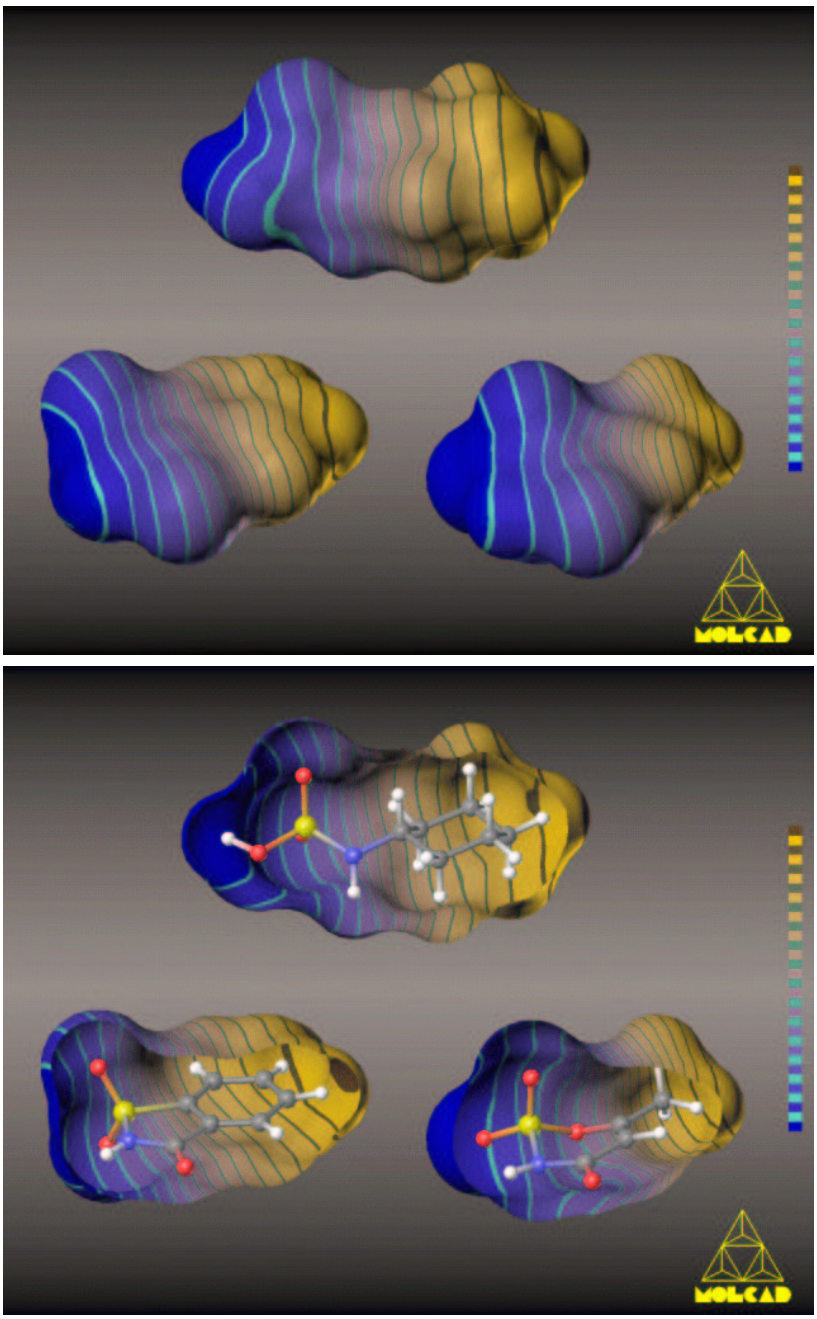


Fig. 2-19. The molecular hydrophobicity profiles (MLP's) in color-coded representation (yellow-brown: hydrophobic, blue: hydrophilic regions) of the sulfamide sweeteners cyclamate (**64**, *upper middle*), saccharin (**65**, *lower left*), and acesulfame (**66**, *lower right*) in closed and opened form. The MLP's are scaled separately to the range of the hydrophobicity values calculated onto the respective contact surfaces cf. Fig. 2-18.

Another striking feature is that hydrophobic and hydrophilic portions of the molecules are on opposite sites, as in the case of sucrose and fructose (cf. above). Moreover, the very same distinctive separation of hydrophilic and hydrophobic areas is observed for the dipeptide sweeteners **67** (aspartame) and **68**, which appear to be quite different in their solid state conformations (ball and stick model insert in

Fig. 2-20), yet foreshadow a basic molecular shape similarity in their contact surfaces (Fig. 2-20, in dotted contours), which fully tallies with the respective MLP's of Fig. 2-21: closely corresponding hydrophobic (yellow-brown) regions of such diverse elements as the aromatic ring of the phenylalanine portion of aspartame, and the sterically constrained tetramethylcyclopentyl group in **68**.

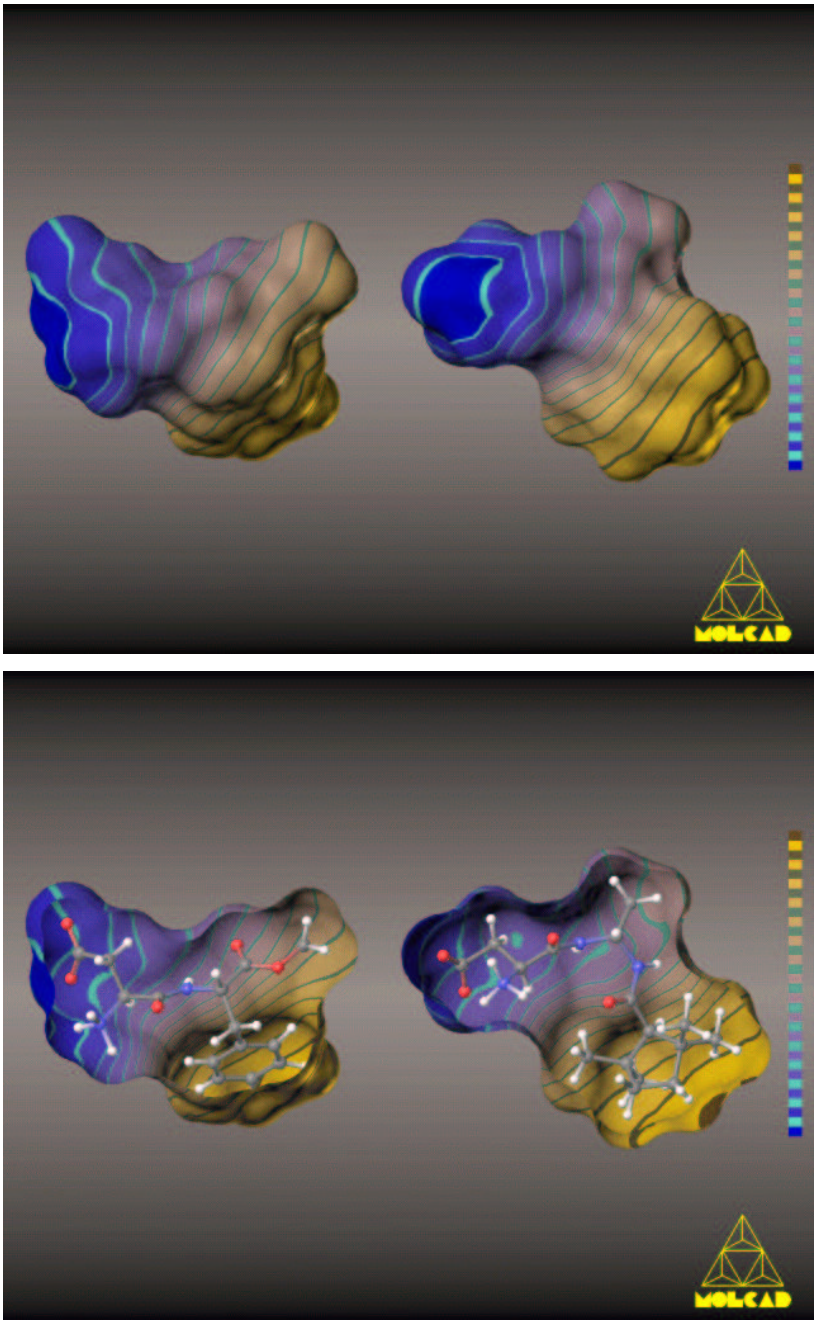


Fig. 2-21. Lipophilicity profiles of the dipeptide sweeteners aspartame (**67**, *left*) and *N*-L-asparty*l*-*N*'-(2,2,5,5-tetramethylcyclopentanyl)-carbonyl-(*R*)-1,1-diaminomethane (**68**, *right*) based on their crystal conformations[112,113].

All of this sustains the notion that the sweet receptor – be it the same for sucrose, fructose, and non-carbohydrate sweeteners or different ones – is quite flexible in adapting to the hydrophobic portion of sweet substances, to the X part (of the tripartite AH-B-X glucophore), which clearly is not a specific position of the molecule, but an entire region. If this hydrophobic area is 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, it can well be imagined that, thereby, the hydrophilic area of the molecule, situated on its opposite site, and likely to contain 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 previously developed.