ELSEVIER

Carbohydrate Research 282 (1996) 25–40

CARBOHYDRATE RESEARCH

On the sweetness properties of aldoses: characterization of molecular active sites by computer simulation

Eduardo I. Howard, J. Raul Grigera *

Instituto de Física de Líquidos y Sistemas Biológicos (IFLYSIB), CONICET, UNLP, and Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, C.C. 565, 1900 La Plata, Argentina

Received 16 December 1994; accepted in final form 7 September 1995

Abstract

We have analyzed the conformation and hydration of β-D-glucopyranose, β-D-galactopyranose, and α- and β-D-mannopyranose, using molecular dynamics simulation. We have focused on the properties of atoms that participate in structures that stimulate the sweetness receptor. From the assignment of the possible molecular centers in the ligand acting as stimuli for a sweet and bitter taste, it was concluded that no hydrophobic G site is active in the aldohexoses studied. The possible participation of a proton donor XH site to explain the bitter taste of β-D-mannopyranose is also proposed.

Keywords: Sweetness; Ligand–receptor interaction; Aldohexoses; Molecular dynamics simulation of sugar conformation

1. Introduction

The sweetness properties of small carbohydrates are of interest both for the food industry and as models of ligand–receptor interaction. Particularly, aldoses have been extensively studied.

It has been established that most of the sweet molecules have a bipolar electrophilic–nucleophilic (AH–B) system that produces hydrogen-bond interaction with the receptor [1]. However, several molecules with similar groups have different sweet-

* Corresponding author. Tel. +54-21-254904; Fax: +54-21-257317.

SSDI 0008-6215(95)00368-1

ness properties, absence of it, or, even more striking, a bitter taste [2]. For the bitter molecules it is believed that the system consists of the same kind of sites as the sweet molecules, but interacting in an inverse order with the AH–B axis of the receptor. At least a third site is necessary to define the molecular orientation. Moreover, it emerges that a geometrical analysis of the molecule alone is not enough to establish its sweetness properties. It is necessary to incorporate the behavior of the molecular hydroxyl groups in solution to show their donor or acceptor character.

Kier [3] has proposed that the presently named site G (or γ), of hydrophobic character, provides the necessary reference for the correct attaching of the molecule to the receptor. Site G is a dispersive site that can be represented by a sphere of radius 0.4 nm. Through the consideration of such site it has been possible to explain, for example, the increase of sweetness properties of halogen-substituted aldohexoses.

The studies of molecular sweetness have been carried out by different techniques: spectroscopy, thermodynamic properties [4,5], molecular mechanics, ab initio methods [6], and, more recently, molecular dynamics simulation for isolated molecules [7] and in solution [8]. This last technique has an advantage over static methods, such as molecular mechanics and ab initio studies, in that it incorporates dynamic behavior producing time-averaged properties. It also provides, when the solvent is incorporated, detailed information concerning the hydration of individual atoms. Under the assumption that the interaction with the receptor does not considerably alter the molecular conformation, it is possible to characterize the donor or acceptor properties of sites. These features make molecular dynamics a useful tool for the proposed studies.

We have performed a molecular dynamics simulation analysis of β-D-glucopyranose, β-D-galactopyranose, and α- and β-D-mannopyranose, focusing on the properties of atoms that are able to participate in structures capable of stimulating the sweetness receptor. Our conclusions support the view that no G site exists in the aldohexoses studied. We also propose the possible participation of a proton donor group (XH). With this assumption it is also possible to explain the bitter taste of β-D-mannopyranose.

2. Methods and calculations

Consideration of sites in aldohexoses.—To start with, we accept the eight-site model of the receptor proposed by Tinti and Nofre [9] that has been successful in devising compounds of high sweetness. In this model four sites are considered of high affinity and four of low affinity. Those named AH, B, G, and D are of high affinity while Y, XH, E_1, and E_2 are of low affinity. The geometry of the model and the characteristics of each site are shown in Table 1.

The site G is considered, as mentioned before, to be of high affinity, and its presence is an indication of a very sweet molecule. The cases of D-tryptophan and cyclamic acid are interesting examples. Only AH, B, and G sites are present in such compounds and their sweetness potencies are, respectively, 35 and 50 times that of sucrose. On the other hand, all sweet aldohexoses are of lower sweetness potency than sucrose. If, as seems likely, they have AH and B sites, the incorporation of a G site should produce a very sweet molecule. From these arguments, it is reasonable to assume that a low-affinity site would be present in the ligand, rather than a high-affinity G site.

Table 1
Average distance (nm) between the eight sites involved in the model of Tinti and Nofre (ref. [9])

Site [a]	AH	B	G	D	Y	XH	E_1
E_2	0.70	0.70	0.45	1.15	0.45	0.30	0.30
E_1	0.80	0.75	0.70	1.25	0.50	0.35	
XH	0.45	0.45	0.55	1.10	0.32		
Y	0.35	0.50	0.45	0.80			
D	0.70	0.90	0.90				
G	0.60	0.80					
B	0.28						

[a] AH and XH correspond to the nuclei of hydrogen atoms belonging to a donor group in the hydrogen bond of the receptor; B, D, Y, E_1, and E_2 correspond to the nuclei of proton acceptors in the hydrogen bond of the receptor, and G represents the center of a hydrophobic group approximately spherical of radius near to 0.4 nm.

The results of Lindley and Birch [10] on the methylation of α-D-glucopyranose show that when monomethylation is carried out in the O-2, O-3, O-4, or O-6 positions, the sweetness potency is not altered significantly. If a G site were involved, the geometry of the site cluster could hardly be adjusted to satisfy the geometric requirements. We face a situation in which switching between different atoms as stimulants of the receptor would be necessary, which is unlikely to occur.

These results cast doubts on the existence of a G site in aldohexoses. The next step is to analyze whether a hydrophobic site, fitting into the site cluster necessary for sweetness stimulation, can be present in aldohexoses.

Computational method.—Molecular dynamics simulations of β-D-glucopyranose, β-D-galactopyranose, and α- and β-D-mannopyranose were done using the GROMOS [11] package in which the equations of motion are integrated using a leap-frog algorithm. Constant temperature (296.16 K) and constant pressure (101325 Pa = 1 atm) were taken. Separate thermal adjustments for solvent and solute were used to avoid local thermal fluctuation, which may be of importance in a run of moderate length. One sugar molecule and 216 water molecules were simulated in a cubic box with average dimensions of 1.892 for β-D-glucose, 1.896 for β-D-galactose, 1.892 for α-D-mannose, and 1.878 nm for β-D-mannose. Tetrahedral geometry was kept by using appropriate improper dihedral potentials. Bond distances were held constant by use of the SHAKE [12] procedure. All data correspond to equilibrium. This condition was established by checking energy and density. All the reported results correspond to averages of over 100-ps simulation time.

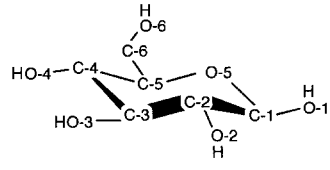
The molecular model.—Crystal positions were used as the starting configuration for carbohydrate molecules. Atom–atom and angular potentials provided by GROMOS were used, but all atoms were explicitly considered (i.e., no united atoms were taken), by contrast with the GROMOS force field in which, for instance, CH and CH_2 are considered as single (united) atoms. The hydrogen potential was taken only as an interacting charge. Charges used were those computed by Brady [13] using ab initio methods and are shown in Table 2. The pyranoid rings were kept in a $^4C_1$ conformation by applying restriction through improper dihedrals. This is done since it has been shown that this is the preferred conformation during equivalent periods of molecular dynamics

Table 2
Partial atom charges

Atom	Charge (e)
C-1	0.35
H-1	0.10
O-1	−0.65
H(O-1)	0.40
C-2	0.15
H-2	0.10
O-2	−0.65
H(O-2)	0.40
C-3	0.15
H-3	0.10
O-3	−0.65
H(O-3)	0.40
C-4	0.15
H-4	0.10
O-4	−0.65
H(O-4)	0.4
C-5	0.10
H-5	0.10
O-5	−0.40
C-6	0.05
Ha-6	0.10
Hb-6	0.10
O-6	−0.65
H(O-6)	0.40

simulations [14]. Therefore, no torsional potentials were applied to the ring. As for the other dihedrals present (pendant groups), we did not apply torsional potentials, since they should arise spontaneously from atom–atom interaction. We have already discussed this case [15]. The water model was SPC/E [16]. Since water was explicitly included, coulombic interactions were computed using a dielectric permittivity equal to unity. Atoms are numbered as shown in Fig. 1.

We have used a geometrical criterion for hydrogen-bond formation. A donor or acceptor atom forms a hydrogen bond when the distance between the acceptor and the hydrogen atom is less than 0.24 nm and the A–H–B angle lies between 145 and 180°.



β -D-glucopyranose

Fig. 1. Representation of β-D-glucopyranose, and the atomic numbering used in the paper.

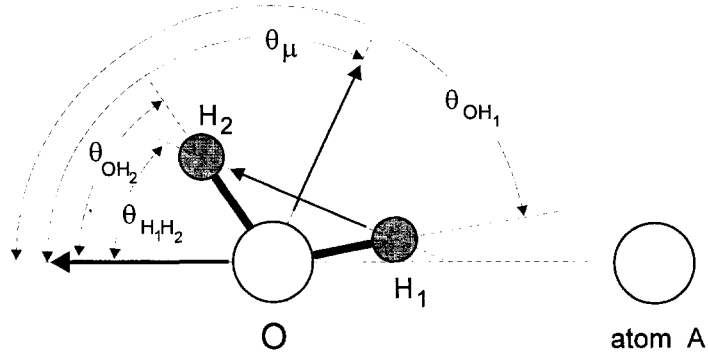


Fig. 2. Angles used for computation of the angular distribution function.

Distribution functions.—To describe the hydration properties around solute atoms and their proton donor or acceptor characteristics, we have computed the radial and angular distribution functions.

Radial distribution functions describe the probability density of finding a given atom (water oxygen, for instance) around the one studied. This allows us to distinguish clearly between an atom having a polar or non-polar character. In the first case we will find a well-defined peak of oxygen located at a distance suitable to form a hydrogen bond.

Angular distribution functions give an indication of the average orientation of the water molecules around a given atom. A description of the angles defined is shown in Fig. 2. The schematic patterns of angular distribution functions for ideal proton acceptor and proton donor are shown in Fig. 3.

3. Results and discussion

As we have described before, the G site is of high affinity and therefore could not be present in molecules of relatively low sweetness power. However, we will consider which groups could be candidates for a G site.

Because of the position of the proton donor and acceptor groups that contribute to AH and B sites, C-6 is a candidate for the G site. However, its geometry is far from optimal. Fig. 4 shows the radial distribution function of water around carbons in glucose. The absence of an oxygen peak is an indication of the non-polar character of a group (water repellency). It can be seen that C-6 has less non-polar character than the others because of the sharp peak near 0.32 nm. Fig. 5 shows the angular distribution function for C-6. In that figure we can see that no clear-cut peaks occur, which indicates that the orientation of water molecules around C-6 is not well defined. A relatively good order is expected for a true hydrophobic atom. This is consistent with the low polar character observed in the radial distribution function. This result is even more unfavorable than the one obtained by Brady for α-D-glucose, in which C-6 is slightly hydrophobic. Moreover, the HO-6 group shows a high hydrophilic character, making the environment more appropriate for a hydrophilic rather than hydrophobic interaction.

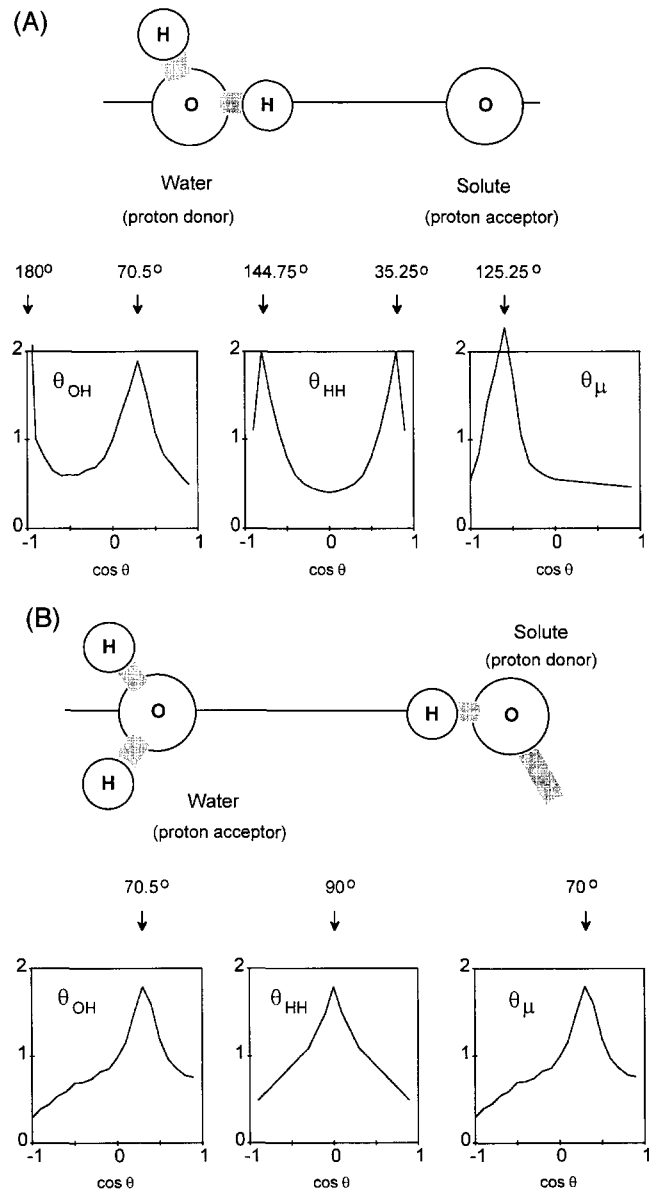


Fig. 3. Schematic patterns of angular distribution function for ideal acceptor and ideal donor: (A) ideal acceptor; (B) ideal donor.

If the positions of the AH and B sites are changed, as would be necessary to account for the methylation experiment, we must look for the G site elsewhere. In this case neither the carbon atoms nor the O-5 atom (of a clear non-polar character) fit into the expected geometry of a G site, even allowing for large tolerances.

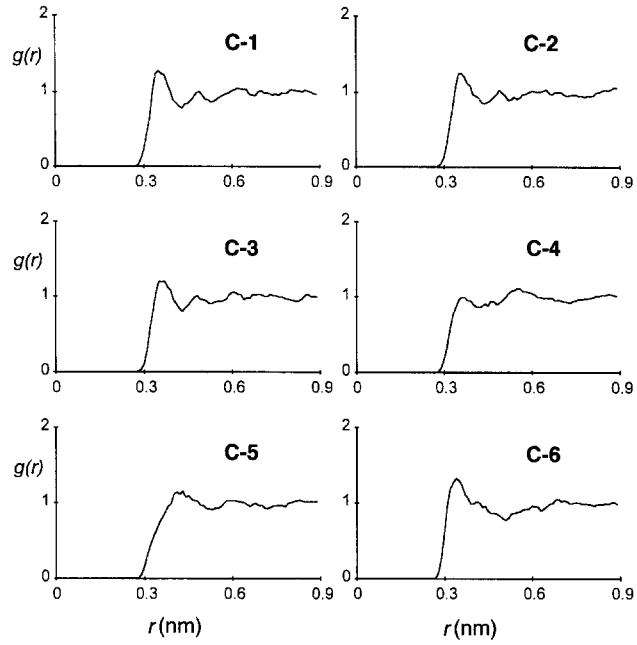


Fig. 4. Radial distribution function of water oxygen around β-D-glucopyranose carbons.

The preceding discussion leads to excluding the occurrence of a G site as an explanation of sweetness in aldohexoses and to a search for another kind of site that correctly accounts for the qualitative and quantitative differences shown by this group of

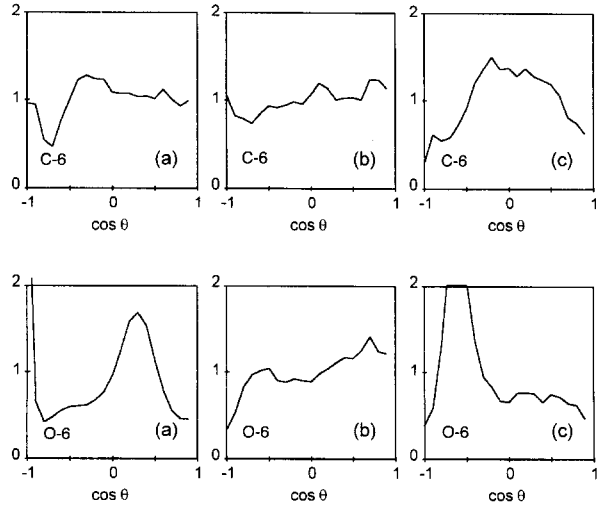


Fig. 5. Angular distribution function of water around β-D-glucopyranose C-6 and O-6. The curve corresponds to the hydration shell with $r < 0.35$ nm: (a) O–H; (b) H–H; (c) μ.

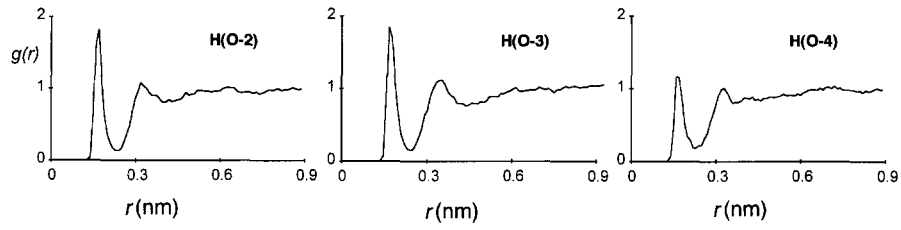


Fig. 6. Radial distribution function of water oxygen around β-D-glucopyranose hydrogens belonging to hydroxyl groups.

molecules in their sweetness properties. We shall accordingly focus our attention on low-affinity sites, of which there are four in these molecules: three are proton acceptor groups (Y, E_1, and E_2) and another is a proton donor group (XH).

We know that β-D-galactopyranose has less sweetness power than β-D-glucopyranose, that α-D-mannopyranose is as sweet as glucose, and that β-D-mannopyranose is bitter. These four molecules cover the different aspects of the problem.

From the analysis of the simulation results, and for geometric and functional reasons, we propose the XH group as a third site involved in the interaction of aldohexoses with the receptor. This site corresponds to a proton donor group and is of low affinity, and its action does not greatly increase the sweetness. According to the model used, it is located at equal distances from the AH and B sites (0.45 nm). Being a proton donor site, its position is that of the hydrogen atom of a hydroxyl group of the monosaccharide.

In all cases we first look for the adjacent pair of hydroxyl groups that correspond to the AH–B system and then for the fit to the third secondary site XH. There are six possible pairs of adjacent hydroxyl groups in each molecule: HO-1–O-2; HO-2–O-1; HO-2–O-3; HO-3–O-2; HO-3–O-4; and HO-4–O-3. In the case of glucose and galactose we disregard HO-1, since their sweetness remains unchanged when we shift from the α to the β anomer.

Our simulation of glucose shows that HO-4 is not a good proton donor in H bonds with water. Fig. 6 shows that the peak of the radial distribution function of the oxygen atoms of water around the hydrogen atom of HO-4 is much lower than, for instance, around the hydrogen atom of HO-3.

Fig. 7 shows a series of plots of the angular distribution function around O-2, O-3, and O-4. It can be seen that the curve corresponding to the function H–H for O-4 lacks the peak at zero that characterizes the existence of water molecules acting as proton acceptors (WO · · · H–O-4). Also, in the curve corresponding to the function μ, the expected peak at ca. 0.7 for a proton donor group is negligible.

Finally, and in agreement with the previous results, we have found that only for 68% of the simulation time are there water molecules in positions suitable to form H bonds with HO-4 as proton donor. This is to be compared with 84% for HO-3 and 80% for HO-2 (Table 3).

Based on this information we can establish that O-4 in β-D-glucopyranose might correspond to a site B, and therefore the adjacent HO-3 to the site AH. This is in good agreement with the simulation results shown above since the AH site is a very good proton donor.

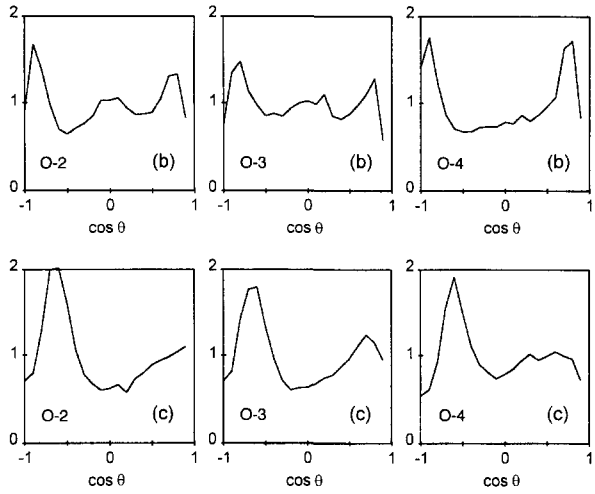


Fig. 7. Angular distribution function of water around β-D-glucopyranose oxygens. The curve corresponds to the hydration shell with $r < 0.35$ nm: (b) H–H; (c) μ.

Table 3
Number of hydrogen bonds between hydroxyl groups of β-D-glucopyranose and water

	HO-2	HO-3	HO-4 [c]
Proton donor [a]	80	84	68
Proton acceptor [b]	3761	3514	3600

[a] Expressed as percentage of simulation time in which the hydroxyl group forms a hydrogen bond.
[b] Total number of hydrogen bonds formed along the simulation.
[c] Corrected to compensate for internal hydrogen bonds with O-6.

In β-D-galactopyranose the situation differs in the sense that HO-4 is a good proton donor. Fig. 8 shows the radial distribution function of water oxygen atoms around hydrogen atoms. For the H of HO-4, a high peak at 0.18 nm and a clear second peak are observed, which denote a clear ordering around the hydrogen. Of these three plots the lower peak corresponds to the H of HO-2.

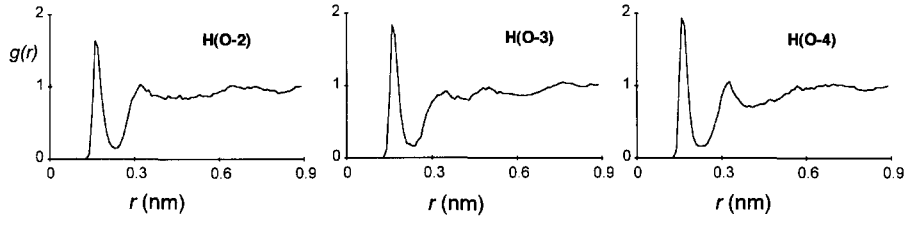


Fig. 8. Radial distribution function of water oxygen around β-D-galactopyranose hydrogens belonging to hydroxyl groups.

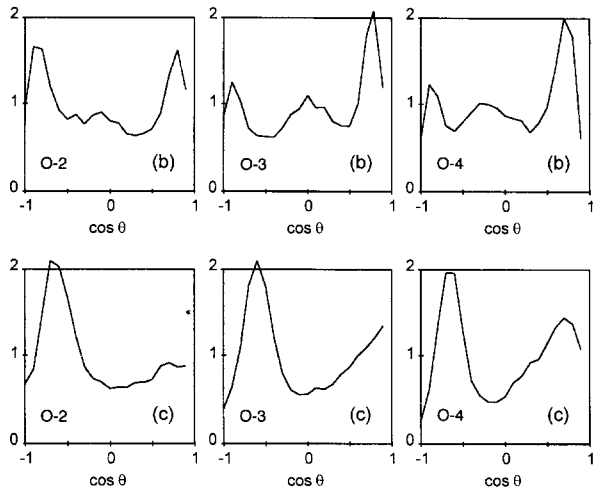


Fig. 9. Angular distribution function of water around β-D-galactopyranose oxygens. The curve corresponds to the hydration shell with $r < 0.35$ nm: (b) H–H; (c) μ.

In Fig. 9 (angular distribution functions, ADF) we see that HO-4 in galactose is again a good proton donor. We find for O-4 and O-3 a peak at the zero value of the plot H–H and also a relatively important peak at 0.7 in the plot of μ, all indicative of a good proton donor. We see that O-2 is now a poor proton donor, but it remains a good acceptor.

Table 4 shows the results of the number of hydrogen bonds for galactose. We can see that HO-2 is the worst proton donor and the best proton acceptor, which makes this group the most sensible option for site B. We therefore have HO-3 for the AH site, as the adjacent hydroxyl group, in agreement with all the results since O-3 is a good proton donor.

From the groups in glucose and galactose capable of taking part in the stimulation of the receptor, not included in the system AH–B, i.e., HO-2 for glucose and HO-4 for galactose, both have good proton donor characteristics and have been considered as an XH site.

In Table 5, we give the resulting distances. We see that glucose fits the expected distances better than galactose, which is in agreement with its larger sweetness power. The larger discrepancies are observed in the distance AH–XH, which may be influenced

Table 4
Number of hydrogen bonds between hydroxyl groups of β-D-galactopyranose and water

	HO-2	HO-3	HO-4
Proton donor [a]	74	81	84
Proton acceptor [b]	4201	4136	3584

[a] Expressed as percentage of simulation time in which the hydroxyl group forms a hydrogen bond.
[b] Total number of hydrogen bonds formed along the simulation.

Table 5
Site positions and distances

	Site			Distances (nm)		
	B	AH	XH	B–AH	B–XH	AH–XH
Model [a]				0.28	0.45	0.45
β-D-Glucose	O-4	HO-3	HO-2	0.33	0.52	0.32
β-D-Galactose	O-2	HO-3	HO-4	0.33	0.47	0.26

[a] Tinti and Nofre model; see Table 1.

by the rotation of both HO groups around the axis of the CO bond. In the presence of the receptor these distances may be stabilized. However, even considering this possibility, it seems scarcely probable that galactose will stimulate the three sites simultaneously, which, as we have said, would explain the low sweetness power.

To test this hypothesis we have simulated α- and β-D-mannopyranose.

Following the same procedure we look for the acceptor site, corresponding to site B. Fig. 10 shows the radial distribution function of water oxygen atoms around the hydrogen atoms of the studied hydroxyl groups. We also include HO-1. In this figure it is observed that the lower peak corresponds to the hydrogen atom of HO-3, the other groups being good proton donors.

Fig. 11 shows the ADF in the neighborhood of the hydroxyl groups studied. In the plot corresponding to H–H, O-3 does not show a peak at zero, nor a peak at 0.7 in the plot μ, indicating that O-3 is a poor proton donor.

Table 6 shows the results of the number of hydrogen bonds between water and α-D-mannopyranose. Again, in agreement with the previous results, we see that the HO-3 group appears as the best acceptor and the worst donor. So we assign O-3 to site B. As adjacent hydroxyl groups, we have HO-4 and HO-2, both good proton donors and, therefore, both suitable for site AH.

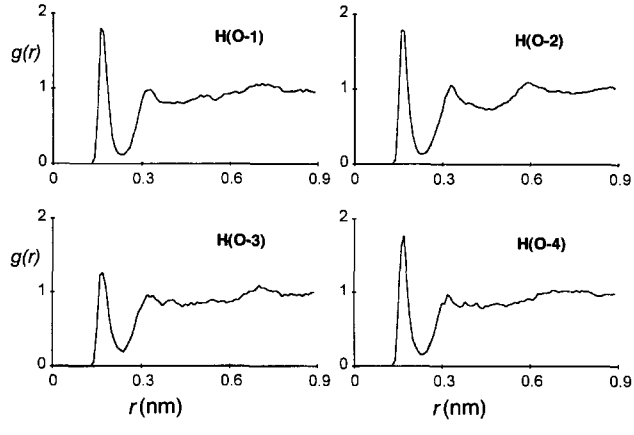


Fig. 10. Radial distribution function of water oxygen around α-D-mannopyranose hydrogens belonging to hydroxyl groups.

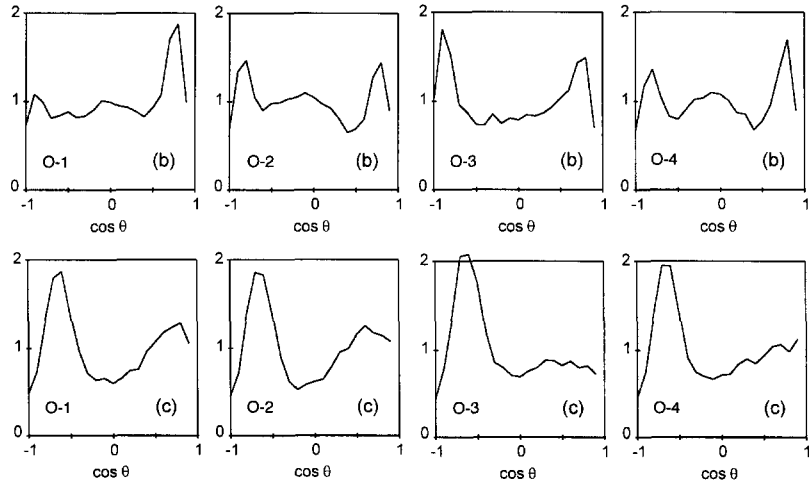


Fig. 11. Angular distribution function of water around α-D-mannopyranose oxygens. The curve corresponds to the hydration shell with $r < 0.35$ nm: (b) H–H; (c) μ.

Table 7 shows the different alternatives for site B. It can be seen that the fit of sets O-3–HO-2–HO-1 and O-3–HO-4–HO-1 for sites B–AH–XH is very good. However, the first option should be disregarded since the hydroxymethyl group of the exocyclic C-6 would penetrate the receptor wall proposed by Shallenberger et al. [17]. As a consequence, the sequence O-3–HO-2–HO-1 in sites B–AH–XH seems to be the best option.

Table 6
Number of hydrogen bonds between hydroxyl groups of α-D-mannopyranose and water

	HO-1	HO-2	HO-3	HO-4
Proton donor [a]	83	83	68	84
Proton acceptor [b]	3344	3158	4065	3200

[a] Expressed as percentage of simulation time in which the hydroxyl group forms a hydrogen bond.
[b] Total number of hydrogen bonds formed along the simulation.

Table 7
Site positions and distances

	Site			Distances (nm)		
	B	AH	XH	B–AH	B–XH	AH–XH
Model [a]				0.28	0.45	0.45
α-D-Mannose	O-3	HO-4	HO-2	0.29	0.33	0.51
	O-3	HO-4	HO-1	0.29	0.49	0.52
	O-3	HO-2	HO-1	0.33	0.49	0.47
	O-3	HO-2	HO-4	0.33	0.29	0.51

[a] Tinti and Nofre model; see Table 1.

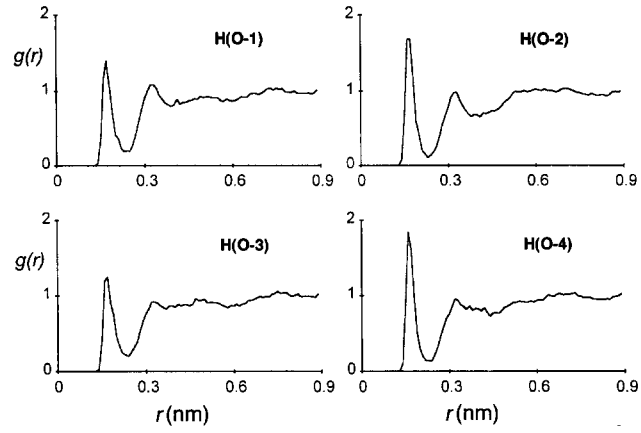


Fig. 12. Radial distribution function of water oxygen around β-D-mannopyranose hydrogens belonging to hydroxyl groups.

Finally, we analyze β-D-mannopyranose in the same way. Fig. 12 shows the RDF for hydrogen atoms in HO-1, HO-2, HO-3, and HO-4 groups. Both H(O-1) and H(O-3) have relatively low peaks.

Fig. 13 corresponds to the ADF. For the H–H plot, the only one that gives a peak at zero value is O-2, but the plot corresponding to μ shows that it is O-1 that presents the worst proton donor characteristic, since besides the lack of a peak at zero it does not have any peak in the positive region of function μ.

Table 8 shows the results for hydrogen bonds. These results confirm that HO-1 is a bad donor and good acceptor, while HO-3 is a bad donor and also a poor acceptor. On

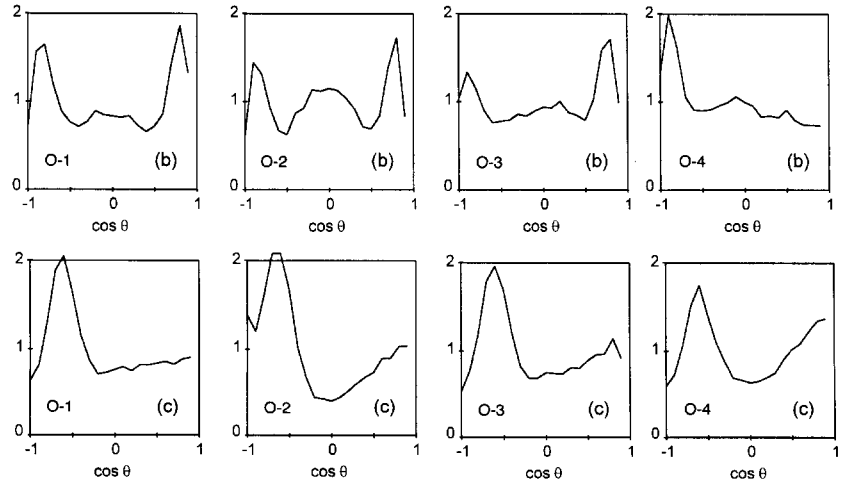


Fig. 13. Angular distribution function of water around β-D-mannopyranose oxygens. The curve corresponds to the hydration shell with $r < 0.35$ nm: (b) H–H; (c) μ.

Table 8
Number of hydrogen bonds between hydroxyl groups of β-D-mannopyranose and water

	HO-1	HO-2	HO-3	HO-4
Proton donor [a]	70	79	70	76
Proton acceptor [b]	3891	4031	3465	2737

[a] Expressed as percentage of simulation time in which the hydroxyl group forms a hydrogen bond.
[b] Total number of hydrogen bonds formed along the simulation.

the contrary, HO-2 — a bad acceptor for the α isomer — is a very good donor and acceptor, while HO-4 is clearly a good donor.

It is important to note the change in the hydration characteristics brought about by the change in stereochemistry at the anomeric carbon. For β-D-mannopyranose it is impossible to choose for the system B–AH–XH the sequence O-3–HO-4–HO-1 as in α-D-mannopyranose, not only because of the changes in the distances but also because of the changes in hydration.

This new situation forces us to consider other alternatives for the groups that can stimulate the receptor. Following the same type of analysis as in the three previous molecules, we should first select HO-1 to occupy site B, consequently HO-2 the site AH, and HO-4 site XH. Table 9 shows the average distances between groups, according to the simulation of β-D-mannopyranose. It can be seen that under the alternative that considers O-1 in site B and HO-4 in site XH, the distance B–XH does not fit the expected geometry.

The hydration characteristics of O-2 allow us to consider it as site B, but in that case we must take HO-3 as AH. This is highly improbable, since in the case of this group having any role at all in the system it would be that of a proton acceptor. We tried the two alternatives with HO-3 as proton acceptor and, therefore, O-3 as site B (Table 9). We see that the sequence O-3–HO-4–HO-2 in sites B–AH–XH may stimulate the sweetness receptor, while the sequence O-3–HO-2–HO-4 in sites B–AH–XH will leave the molecule behind the receptor wall.

If we take the group O-3–HO-2–HO-4 but in sites AH–B–XH, we see that it may stimulate a bitter receptor. In this way β-D-mannopyranose has the possibility of interacting with a sweet or a bitter receptor, from which we may expect both tastes.

Table 9
Site positions and distances

	Site			Distances (nm)		
	B	AH	XH	B–AH	B–XH	AH–XH
Model [a]				0.28	0.45	0.45
β-D-Mannose	O-1	HO-2	HO-4	0.31	0.61	0.46
	O-3	HO-4	HO-1	0.31	0.31	0.46
	O-3	HO-2	HO-1	0.31	0.31	0.46

[a] Tinti and Nofre model; see Table 1.

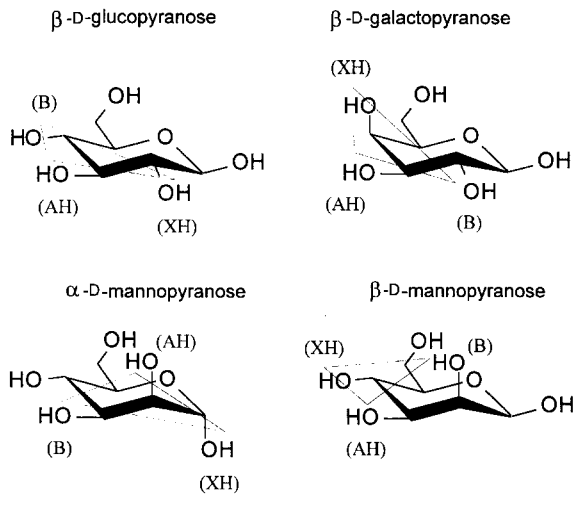


Fig. 14. Representation of the compounds studied, and the possible interaction groups.

However, we know [18] that a bitter substance, even in concentrations several times lower than the sweet one, has the effect of a large depression of sweetness. As a consequence we expect a bitter molecule, as experimentally observed. Fig. 14 shows the representation of the compound studied, and the possible interaction groups.

4. Conclusions

Our molecular dynamics simulation of non-substituted aldohexoses shows the lack of a hydrophobic site compatible with the geometry proposed by Tinti and Nofre [9] to be considered as a site G. Therefore, we searched for alternatives to explain the properties of a number of aldohexoses as sweet molecules. We have searched for a low-affinity site that accounts for the different sweetness properties of aldohexoses, including the fact that β-D-mannopyranose is bitter.

We have found that, in the molecules considered, the system B–AH–XH satisfied the requirements. Particularly in α-D-mannopyranose, the choice involves the HO-1 group, which explains the differences in behavior between α and β anomers.

One of the main drawbacks of the model presented here is that it is based on an estimation of the site geometry; though elaborate, this cannot define precisely the orientation of interacting groups. Since hydrogen bonds are sensitive to the orientation of acceptor and donor, the lack of details prevents us from drawing quantitative conclusions.

In spite of the obvious simplifications made, we consider that the present analysis allows a novel interpretation of the geometry of the sites involved in the sweetness–bitterness of aldohexoses.

Acknowledgements

This work was partly supported by the Consejo Nacional de Investigaciones Cientificas y Tecnicas of Argentina (CONICET). E.I.H. is a Fellow of CONICET, and J.R.G. a member of the Carrera de Investigador of the same Institution. We thank Professors H.J.C. Berendsen and W.F. van Gunsteren for allowing the use of the GROMOS package.

References

[1] R.S. Shallenberger and T.E. Acree, *Nature (London)*, 216 (1967) 480–482
[2] J. Solms, *J. Agric. Food. Chem.*, 17 (1969) 686
[3] L.B. Kier, *J. Pharm. Sci.*, 61 (1972) 1394–1397.
[4] M. Mathlouthi and A.M.Seuvre, *J. Chem. Soc., Faraday Trans. 1*, 84 (1988) 2641–2650.
[5] G.G. Birch and S. Shamil, *J. Chem. Soc., Faraday Trans. 1*, 84 (1988) 2635–2640.
[6] F. Lelj, T. Tancredi, P.A. Temussi, and C. Toniolo, *J. Am. Chem. Soc.*, 98 (1976) 6669–6674.
[7] M. Castiglione-Morelli, F. Lelj, F. Naider, M. Tallon, T. Tancredi, and P.A. Temussi, *J. Med. Chem.*, 33 (1990) 514–520.
[8] E.I. Howard and J.R. Grigera, *J. Chem. Soc., Faraday Trans.*, 88 (1992) 437–441.
[9] J.M. Tinti and C. Nofre, *ACS Symp. Ser.*, 450 (1991) Chapter 15.
[10] M.G. Lindley and G.G. Birch, *J. Sci. Food. Agric.*, 26 (1975) 117.
[11] W.F. van Gusteren and H.J.C. Berendsen, GROMOS, 1987; from BIOMOS, Bv. Nijenborg 4, 9747 AG Groningen, The Netherlands.
[12] J.P. Ryckaert, G. Ciccotti, and H.J.C. Berendsen, *J. Comput. Phys.*, 23 (1977) 327–335.
[13] J.W. Brady, *J. Am. Chem. Soc.*, 108 (1986) 8153–8160; *Carbohydr. Res.*, 165 (1987) 306–312.
[14] J.W. Brady, *J. Am. Chem. Soc.*, 111 (1989) 5165–5173.
[15] J.R. Grigera, *J. Chem. Soc., Faraday Trans. 1*, 84 (1988) 2603–2608; J.R. Grigera, in A. French and J.W. Brady (Eds.), *Computer Modelling of Carbohydrate Molecules*, American Chemical Society, Washington, DC, 1990.
[16] H.J.C. Berendsen, J.R. Grigera, and T.P. Straatsma, *J. Phys. Chem.*, 91 (1987) 6269–6271.
[17] R.S. Shallenberger, T.E. Acree, and C.Y. Lee, *Nature (London)*, 221 (1969) 555–556.
[18] E. Lindsay, in O. Fennema (Ed.), *Food Chemistry*, 2nd ed., Dekker, New York, 1985, p 590.