Abstract: The conformational behaviour of naturally occurring ketohexoses has been revealed in a supersonic expansion by Fourier transform microwave spectroscopy coupled with a laser ablation source. Three, two and one conformers of D-tagatose, D-psicose and L-sorbose, respectively, have been identified by their rotational constants extracted from the analysis of the spectra. Singular structural signatures involving the hydroxyl groups OH\(_{17}\) and OH\(_{12}\) have been disentangled from the intricate intramolecular hydrogen bond networks stabilising the most abundant conformers. The present results place the old Shallenberger and Kier sweetness theories on a firmer footing.

Introduction

Human beings feel attracted to food owing to its flavours and the pleasure experienced when tasting them. The sense of taste, located in the oral cavity, is able to recognize up to five different tastes in food: salty, sour, bitter, sweet and umami (from the Japanese umai; meaning delicious). Perceiving salty and sour tastes is due to the presence of protons and ions. Thus, there is no relation with the structure of the substance. In contrast, sensing the latter three flavours (bitter, sweet and umami) is due to a response induced by chemoreceptors in the taste bud which recognize specific molecular structures. Enquiry into these taste receptors has identified the structures responsible for perceiving each flavour,\(^{[1]}\) taste receptors (TRB), which are members of G-protein-coupled receptors (GPCRs) are present in tongue and palate epithelia.\(^{[2]}\) Particularly, within this TRB group, type T2R taste receptors recognize bitterness whereas sweetness and umami are detected by type T1R; T1R1 and T1R3 are assigned to umami and T1R2 and T1R3 to sweetness.\(^{[3–5]}\) Therefore, only two receptors are responsible for recognising all of the many and diverse sweeteners that exist. In order to account for all of them, these receptors have been conjectured to contain different binding sites, each responsible for detecting sweeteners of similar sizes. Thus, all small-size sweeteners, like monosaccharides or amino acids, are due to interactions with the same receptor site.\(^{[6]}\) Nonetheless, not all similar size molecules are equally sweet. This therefore raises the question concerning which structural characteristics endow sweeteners with their flavour, while other substances with similar structures are not sweet, in other words, what is the relation between sweetness and structure?

Since early last century, abundant research has addressed the link between sweetness and the structure of sweeteners.\(^{[7]}\) Several studies have assigned the sweet properties of a molecule to the presence of particular pairs of functional groups,\(^{[8, 9]}\) while others have assigned it to a specific disposition of an atom.\(^{[2–10]}\) Yet, none of these theories has been able to offer a unified explanation regarding the sweetness–structure relation until Shallenberger and Acree’s proposal.\(^{[11, 12]}\) They observed that the hydrogen bonds of sweet molecules strongly affected their sweetness. Under these circumstances, they stated that the degree of sweetness depends on the strength of two H-bonds by which the sweetener is bound to the sweet receptor (Figure 1a). They established that one of the two electronegative atoms might act as proton donor (AH) in the hydrogen bond interaction and the other as acceptor (B). These two forms group what is called the glucophore, which generally refers to the part of the sweetener that interacts with the sweet receptor. A third binding site was later proposed by Kier, the γ-site, forming the “sweetness triangle” (Figure 1b).\(^{[13–15]}\) This new γ-site might interact with the receptor via hydrophobic or van der Waals’ interaction. Nonetheless, the γ-site was postulated as unnecessary. Indeed, it was stated that it merely enhances the sweet flavour depending on the γ-AH-B distance.\(^{[16]}\) The role of the hydrophobic interactions has been also discussed by Simons et al.\(^{[17]}\) in terms of carbohydrate molecular recognition at aromatic protein binding sites by creating molecular complexes between monosaccharides and tolune.

Evolution of sweetness theories have continued with the introduction of the multipoint attachment theory. Tinti et al. proposed that, besides the complementary sites for the AH-B-γ triangle, the sweet receptor must contain at least five other linking sites where the sweetener can interact, making eight sites in all.\(^{[18]}\) However, the glucophore in the sweetener need not contain the analogous eight binding sites, although the greater the number of interactions, the sweeter the substance. To
Numerous studies on \( \text{C}2\text{C} \) have recently been placed in the gas phase by laser conformer (98\%) stabilised by an network hydroxyl pairs are suitable candidates to be assigned as As confirmed in our study of intramolecular H-bonds in acounter-

2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim pyranoside ring configur-

ation (Figure 2). In the following sections, we analyse their rotational spectrum in order to reveal their most stable structures characterised in the isolation conditions of supersonic expansion. The results of this conformational analysis are used to evaluate the theories proposed to date concerning sweetness. Common structural signatures that might be correlated to their sweetness can shed some light on identifying the glucophore.

The food industry is currently looking to design tasty new substances, not only maintaining a sweet taste but also avoiding undesirable health effects. Consequently, there is keen interest in identifying the structural requirements that a sweet substance must contain in order to plan the synthesis of new artificial sweeteners.

**Results**

\( \text{D-Tagatose} \)

\( \text{D-Tagatose, the C-4 epimer of } \text{D-fructose, is a healthy sweetener with antidiabetic properties.} \)

In its crystalline form, \( \text{D-tagatose} \) exists in the \( \alpha \) form with a \( \text{C}2 \) pyranoside ring configuration (Figure 3). As confirmed in our study of \( \text{D-fructose} \) and in the results of other related sugars, the intercon-

![Image of sweetness theories](Figure 1)

**Figure 1.** Representation of the sweetness theories. a) Shallenberger’s theory: circular area represents the glucophore of the sweetener. b) Diagram of the “sweetness triangle” in the sweetener.

![Image of D-fructose conformers](Figure 2)

**Figure 2.** The two observed conformers of \( \text{D-\text{-fructopyranose}} \) showing the intramolecular hydrogen-bond networks and the glucophore unit.
version between the α and β anomers should not occur during vaporisation, since it is a solvent-mediated reaction. Therefore, its 6 to 12 GHz broad-band rotational spectrum, shown in Figure 4, should be attributed to the α-α-tagatose species as well as the photofragmentation products common to other sugars.

After removing these decomposition lines (Figure 4), pairs of strong a-type R-branch ($J + 1_{0_{a},1_{a}}$) to $J_{0_{a},1_{a}}$ and ($J + 1_{1_{a},1_{a}}$) to $J_{1_{a},1_{a}}$ rotational transitions were easily identified and ascribed to a first rotameric species, labelled as I. Following iterative fittings and predictions, b- and c-type R-branch transitions were assigned and measured for this rotamer. A total of 63 transitions (Table S1 in the Supporting Information) were submitted to rigid rotor analysis leading to the rotational constants listed in the first column of Table 1.

Once the strong lines of rotamer I were removed from the broad-band spectrum, deeper insights into the low intense background led to the assignment of very weak rotational transitions (see inset of Figure 4) belonging to rotamers II and III. For rotamer II, 22 a- and c-type R-branch transitions were measured whereas for rotamer III only 16 a-type were observed (Tables S2–S3 in the Supporting Information). Rigid rotor analysis for each rotamer led to the rotational constants also listed in Table 1.

Experimental values of the rotational constants of rotamers I and II nicely match with those predicted by ab initio calculations (see Table 1 and Table S4 in the Supporting Information) for conformers $\alpha$ C$_5$ g + s and $\alpha$ C$_5$ g + cc (see ref. [39] and footnote of Table 1 for labelling). These are stabilised by intramolecular H-bonds between vicinal hydroxyl groups forming small networks, strongly reinforced by sigma-hydrogen bond cooperativity. In both conformers, the axial orientation of the hydroxyl groups OH$_{1(2)}$ and OH$_{3(5)}$ disrupts any plausible H-bond network over the entire molecule. Thus, as shown in Figure 5, two split H-bond networks stabilise both conformers. They only differ in the clockwise (cl) or counter-clockwise (cc) orientation of the network involving the OH$_{1(2)}$, OH$_{4(5)}$ and OH$_{3(5)}$ hydroxyl groups. This does not significantly affect their rotational constant values but does drastically alter the $\mu_0$ dipole moment component value, which changes from 1.7 D to near zero when passing from the $\alpha$ C$_5$ g + cl conformer to $\alpha$ C$_5$ g + cc. On this basis, rotamer I must be conformer $\alpha$ C$_5$ g + cl and rotamer II conformer $\alpha$ C$_5$ g + cc, since b-type transitions are not observed for rotamer II. The values of the rotational constants of rotamer III allowed conclusive identification of conformer $\alpha$ C$_5$ t cc. It only exhibits a network of four cooperative OH$_{1(2)}$–OH$_{4(5)}$–OH$_{3(5)}$–OH$_{5(2)}$ hydrogen bonds in a counter-clockwise (cc) arrangement favoured by the trans configuration of the hydroxymethyl group.

As proof of the consistency of the overall assignment, a scale factor ranging from 0.991 to 0.997 makes the theoretical values for conformers $\alpha$ C$_5$ g + cl, $\alpha$ C$_5$ g + cc and $\alpha$ C$_5$ t cc concur with the experimental values for rotamers I, II and III, respectively (Table 1). The population distribution of these species, obtained from the relative intensities of rotational transitions, is estimated to be: $\alpha$ C$_5$ g + cl, 72%; $\alpha$ C$_5$ g + cc, 22%; and $\alpha$ C$_5$ t cc, 6%, in accordance with the predicted relative energies.

D-Psicose

D-Psicose, also known as D-allulose or D-ribo-2-hexulose, is the C-3 epimer of D-fructose. Although it is a rare sugar, it has attracted much attention for its promising health properties as a no-energy sweetener. In crystalline phase, D-psicose is stabilised in its β form with a C$_5$ pyranoside ring configuration (Figure 3). Its laser ablated broad-band rotational spectrum (Figure 6) was analysed following the same procedure as described above. After removing known photofragmentation lines, it was easy to identify intense pairs of b-type R-branch progressions ($J + 1_{1_{b},1_{b}}$) to $J_{1_{b},1_{b}}$ and ($J + 1_{1_{c},1_{c}}$) to $J_{1_{c},1_{c}}$ ($J$ ranging from 4 to 7), as belonging to a first rotamer I. Neither a- nor c-type transitions were observed for this species.

As shown in the inset of Figure 6, a detailed inspection of the spectrum revealed the existence of other very weak b-type R-branch progressions close to those of rotamer I. Once their assignment through an isotopic species had been ruled out,
they were attributed to a second rotamer II. As with rotamer I, a- and c-type transitions were predicted but not found. Rigid rotor analysis \[38\] of the measured lines for rotamers I and II, listed in Tables S5–S6 in the Supporting Information, yielded the set of rotational constants displayed in the first and third columns of Table 2. No other weak transitions attributable to other species remain in the broad-band spectrum.

When identifying the observed rotamers, we first took into consideration the very close values of their rotational constants. As for rotamers I and II of d-tagatose, this fact strongly indicates that both species must belong to conformers with the same skeletal frame but with a different orientation of their intramolecular H-bond network. On account of the values of their rotational constants and electric dipole moment components, rotamers I and II are only consistent with those predicted for the two lowest energy conformers \[\beta \ 5_{g}^{\circ} cc\] and \[\beta \ 5_{g}^{\circ} cl\] (Table S7 in the Supporting Information), also listed in Table 2 for comparison.

As shown in Figure 7, each confor-

### Table 1. Experimental and predicted rotational parameters for the observed conformers of d-tagatose.

<table>
<thead>
<tr>
<th>Rotamer</th>
<th>(a_{i}C_{i} g + c^{[\text{i}]})</th>
<th>(a_{i}C_{j} g + cc)</th>
<th>(a_{i}C_{k} \pm cc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1627.8919(33)</td>
<td>1642.359(24)</td>
<td>1636.6</td>
</tr>
<tr>
<td>II</td>
<td>698.29606(15)</td>
<td>699.11441(25)</td>
<td>701.9</td>
</tr>
<tr>
<td>III</td>
<td>588.40040(10)</td>
<td>591.15555(28)</td>
<td>592.3</td>
</tr>
<tr>
<td>(\nu_{i}) [MHz]</td>
<td>63</td>
<td>63</td>
<td>63</td>
</tr>
<tr>
<td>(N_{e}) [kHz]</td>
<td>63</td>
<td>63</td>
<td>63</td>
</tr>
<tr>
<td>(\Delta E_{e}^{[\text{cm}^{-1}]})</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

[a] Ab initio calculations (MP2/6-311 + G(d,p)). [b] \(A\), \(B\) and \(C\) are the rotational constants; \(|\mu_{i}|\), \(|\mu_{j}|\) and \(|\mu_{k}|\) are the absolute values of the electric dipole moment components. [c] The rms deviation of the fit. [d] Number of fitted rotational transitions. [e] Relative electronic energies calculated at MP2/6-311 + G(d,p) level of theory. [f] Standard error in parenthesis in the units of the last digit. [g] Experimental observation of the \(a\), \(b\) and \(c\)-type spectrum. [h] \(\alpha\) or \(\beta\) denote the anomere type. \(5_{g}^{\circ}\) or \(5_{c}^{\circ}\) refer to pyranose ring configuration. The symbols \(g\) (\(-60^\circ\)), \(g\) (\(+60^\circ\)) and \(t\) (\(180^\circ\)) describe the values of the torsion angle \(O_{1}-C_{1}-C_{2}-O_{2}\), which determines the orientation of the hydroxymethyl group. The last label "cc" and "cc" describes the clockwise and counter-clockwise arrangements, respectively, of the intramolecular hydrogen bond networks involving hydroxyl groups \(OH_{\alpha},\ OH_{\beta}\) and \(OH_{\gamma}\).
The intramolecular hydrogen bond networks; one in common OH(2)···OH(1)···O-ring and, as anticipated, a counter-clockwise (cc) OH(5)···OH(4)···OH(3)···OH(5) and a clockwise (cl) OH(1)···OH(4)···OH(3)···OH(1) cyclic H-bond network.

This does not appreciably affect the values of their rotational constants, and does not allow any discrimination between them to be made. In addition, contrary to what was observed in d-tagatose, the values of the dipole-moment components are not altered enough when passing from conformer b(2)C(5)g@cc to b(2)C(5)g@cl, in accordance with the experimental observations of b-type R-branch transitions for both rotamers. It could be a reasonable assignment to ascribe rotamer I to conformer b(2)C(5)g@cc and rotamer II to conformer b(2)C(5)g@cl taking into account the relative intensities of the measured rotational transitions which give rise to a population distribution of 60 and 40%, respectively, in accord with ab initio calculations.

### Table 2. Experimental and predicted rotational parameters for the observed conformers of d-psicose.

<table>
<thead>
<tr>
<th>Rotamer</th>
<th>A (MHz)</th>
<th>B (MHz)</th>
<th>C (MHz)</th>
<th>[μa]_∥</th>
<th>[μa]_⊥</th>
<th>[μb]_∥</th>
<th>[μb]_⊥</th>
<th>[μc]_∥</th>
<th>[μc]_⊥</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1626.7540(38)</td>
<td>723.03579(12)</td>
<td>660.98104(16)</td>
<td>0.4</td>
<td>-</td>
<td>0.2</td>
<td>-</td>
<td>0.0</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>1626.75480(84)</td>
<td>724.68772(41)</td>
<td>661.03584(48)</td>
<td>1.4</td>
<td>observed</td>
<td>0.7</td>
<td>observed</td>
<td>0.0</td>
<td>-</td>
</tr>
</tbody>
</table>

[a] Ab initio calculations (MP2/6-311++G(d,p)). [b] A, B and C are the rotational constants; [μa], [μb] and [μc] are the absolute values of the electric dipole moment components. [c] The rms deviation of the fit. [d] Number of fitted rotational transitions. [e] Relative electronic energies calculated at the MP2/6-311++G(d,p) level of theory. [f] Standard error in parenthesis in the units of the last digit. [g] Experimental observation of the a-, b- and c-type spectrum.
l-Sorbose

l-Sorbose, the C-5 epimer of α-fructose, is as sweet as sucrose. Its main use is not as sweetener, whereas as intermediate in the industrial synthesis of vitamin C.[43] In crystalline phase, l-sorbose is found in the α anomer in the most stable \( ^{\text{C}}\text{C}_5 \) pyranoside ring configuration[44] (Figure 3). Its laser ablated broad-band rotational spectrum was found to be dominated by decomposition lines. After exhaustive detailed searches, a weak progression of a-type R-branch \((J+1)_{J_1J_2} \rightarrow J_{J_1J_2} \) and \((J+1)_{J_1J_2} \rightarrow J_{J_1J_2} \) pairs of transitions ranging from \( J=4 \) to \( J=8 \) was revealed (Figure 8).

Neither b- nor c-type transitions were observed for this species. No lines belonging to other species could be identified. A total of 34 rotational transitions (Table S8 in the Supporting Information) were analysed to derive the rigid rotor rotational constants values shown in Table 3. These are only compatible with those predicted ab initio for conformer \( \alpha \ \ ^{\text{C}}\text{C}_5 \ g- \ cc \) (see Table S9 in the Supporting Information), also listed in Table 3 for comparison. Identification is reinforced by the fact that only weak a-type transitions are observed, in accordance with the predicted values of the electric dipole moment components for this conformer.

The \( \alpha \ \ ^{\text{C}}\text{C}_5 \ g- \ cc \) conformer of l-sorbose is stabilised by a five-cooperative intramolecular hydrogen bond network \( \text{OH}_{\text{B}(1)} \cdots \text{OH}_{\text{B}(1)} \cdots \text{OH}_{\text{B}(1)} \cdots \text{OH}_{\text{B}(1)} \cdots \text{O}_{\text{B}} \) (Figure 9), in a counter-clockwise arrangement, spread over the entire molecule. The observed network shows a strong analogy to that of α-fructose,[26] with the sole exception of the disposition of the \( \text{OH}_{\text{B}(1)} \) which is in axial disposition in α-fructose.

Discussion

In light of this new experimental information regarding the conformational behaviour of ketohexoses, theories explaining sweetness in terms of structure can be revisited in order to discuss their agreement with the present conformational results. Looking at the panel of the conformational panorama of ketohexoses (Figure 10), it is easy to realize that all the most abundant species of ketohexoses: \( \beta \ \ ^{\text{C}}\text{C}_5 \ g- \ cc \) (98%) for α-fructose, \( \alpha \ \ ^{\text{C}}\text{C}_5 \ g+ \ cc \) (72%) and \( \alpha \ \ ^{\text{C}}\text{C}_5 \ g \) (22%) for α-tagatose, \( \beta \ \ ^{\text{C}}\text{C}_5 \ g- \ cc \) (60%) and \( \beta \ \ ^{\text{C}}\text{C}_5 \ g \) (40%) for β-piscose and \( \alpha \ \ ^{\text{C}}\text{C}_5 \ g- \ cc \) for l-sorbose, show the same intramolecular H-bond network \( \text{OH}_{\text{B}(1)} \cdots \text{OH}_{\text{B}(1)} \cdots \text{O}_{\text{B}} \) (see dashed lines in Figure 10). Despite their distinct \( ^{\text{C}}\text{C}_5 \), or \( ^{\text{C}}\text{C}_5 \) ring configurations, the anemonic \( \text{OH}_{\text{B}(1)} \) is always in axial orientation while the \( \text{OH}_{\text{B}(1)} \) points towards the \( \text{O}_{\text{B}} \). All the above conformers share the common conformational signature \( \text{OH}_{\text{B}(1)} \cdots \text{O}_{\text{B}} \), which could be ascribed to the AH and B sites of glucophore, thus concurring with Shallenberger-

![Figure 7](image-url) l-Sorbose, the C-5 epimer of α-fructose, is as sweet as sucrose. Its main use is not as sweetener, whereas as intermediate in the industrial synthesis of vitamin C.[43] In crystalline phase, l-sorbose is found in the α anomer in the most stable \( ^{\text{C}}\text{C}_5 \) pyranoside ring configuration[44] (Figure 3). Its laser ablated broad-band rotational spectrum was found to be dominated by decomposition lines. After exhaustive detailed searches, a weak progression of a-type R-branch \((J+1)_{J_1J_2} \rightarrow J_{J_1J_2} \) and \((J+1)_{J_1J_2} \rightarrow J_{J_1J_2} \) pairs of transitions ranging from \( J=4 \) to \( J=8 \) was revealed (Figure 8).

Neither b- nor c-type transitions were observed for this species. No lines belonging to other species could be identified. A total of 34 rotational transitions (Table S8 in the Supporting Information) were analysed to derive the rigid rotor rotational constants values shown in Table 3. These are only compatible with those predicted ab initio for conformer \( \alpha \ \ ^{\text{C}}\text{C}_5 \ g- \ cc \) (see Table S9 in the Supporting Information), also listed in Table 3 for comparison. Identification is reinforced by the fact that only weak a-type transitions are observed, in accordance with the predicted values of the electric dipole moment components for this conformer.

The \( \alpha \ \ ^{\text{C}}\text{C}_5 \ g- \ cc \) conformer of l-sorbose is stabilised by a five-cooperative intramolecular hydrogen bond network \( \text{OH}_{\text{B}(1)} \cdots \text{OH}_{\text{B}(1)} \cdots \text{OH}_{\text{B}(1)} \cdots \text{OH}_{\text{B}(1)} \cdots \text{O}_{\text{B}} \) (Figure 9), in a counter-clockwise arrangement, spread over the entire molecule. The observed network shows a strong analogy to that of α-fructose,[26] with the sole exception of the disposition of the \( \text{OH}_{\text{B}(1)} \) which is in axial disposition in α-fructose.

![Figure 8](image-url) Broad-band rotational spectrum of l-sorbose obtained by LA-CP-FTMW. Decomposition lines have been removed from the spectrum in order to observe the very weak a-type R-branch \((J+1)_{J_1J_2} \rightarrow J_{J_1J_2} \) and \((J+1)_{J_1J_2} \rightarrow J_{J_1J_2} \) pairs of rotational progressions ranging from \( J=4 \) to \( J=8 \) of the observed rotamer.

![Figure 9](image-url) The observed conformer of α-l-sorbose showing the intramolecular H-bond network in the counter-clockwise (cc) arrangement.

Table 3. Experimental and predicted\(^{\text{[a]}}\) rotational parameters for the observed conformers of l-sorbose.

<table>
<thead>
<tr>
<th>Rotamer</th>
<th>( \alpha \ \ ^{\text{C}}\text{C}_5 \ g- \ cc )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Delta ) [MHz](^{\text{[b]}})</td>
<td>1525.5027(80)</td>
</tr>
<tr>
<td>( \beta ) [MHz]</td>
<td>722.08428(33)</td>
</tr>
<tr>
<td>( C ) [MHz]</td>
<td>556.05153(30)</td>
</tr>
<tr>
<td>(</td>
<td>J_1</td>
</tr>
<tr>
<td>(</td>
<td>J_2</td>
</tr>
<tr>
<td>(</td>
<td>J_3</td>
</tr>
<tr>
<td>( \Delta \mu ) [kHz]</td>
<td>8.4</td>
</tr>
<tr>
<td>( K^{\text{[d]}} )</td>
<td>34</td>
</tr>
</tbody>
</table>

\(^{[a]}\) Ab initio calculations (MP2/6-311+ + G(d,p)). \(^{[b]}\) \( \Delta \), \( \beta \) and \( C \) are the rotational constants; \( |J_1| \), \( |J_2| \) and \( |J_3| \) are the absolute values of the electric dipole moment components. \(^{[c]}\) The rms deviation of the fit. \(^{[d]}\) Number of fitted rotational transitions. \(^{[e]}\) Standard error in parenthesis in the units of the last digit. \(^{[f]}\) Experimental observation of the a-, b- and c-type spectrum.
The reverse identification of AH as OH has been characterised for a “sweetness triangle” observed conformers for all ketohexoses. Dashed lines indicate as acceptor (B), concurring with Shallenberger’s old proposal in accordance with the aforementioned sweetness theory. Thus, based on the common conformational signatures of ketohexoses, the “sweetness triangle” formed by OH/\(\alpha\)-OH/\(\beta\)-OH and C\(_{10}\) (green triangles in Figure 10) is present in all stable conformers of ketohexoses in line with Kier’s theory.

The degree of sweetness of ketohexoses can also be considered in light of the present results. It is well known that \(\alpha\)-fructose is twice as sweet as \(\alpha\)-sorbose. Surprisingly, the conformational behaviour observed for both sugars, shown in Figure 10a and d, revealed extraordinary similarities. Hence, the most abundant \(\beta\) C\(_2\) g cc and \(\alpha\) C\(_2\) g cc species of \(\alpha\)-fructose and \(\alpha\)-sorbose are stabilised by the same H-bond network \(\text{OH}_\text{gest} \cdots \text{OH}_\text{gest} \cdots \text{OH}_\text{gest} \cdots \text{O}_\text{ring}\) spread over the entire molecule. Some authors have rationalised this difference in sweetness in terms of the existence of an intramolecular H-bond between the \(\text{OH}_\text{gest}\) and \(\text{O}_\text{ring}\) in \(\beta\)-fructopyranose and its absence in \(\alpha\)-L-sorbo.pyranose. However, no \(\text{OH}_\text{gest} \cdots \text{O}_\text{ring}\) hydrogen bond interactions are observed in any of the observed conformers of the ketohexoses. Speculating with the multipoint attachment theory, which also assume the presence of AH, B and \(\gamma\) sites in the glucophore, the distinct axial/equatorial orientation of the \(\text{OH}_\text{gest}\) in the \(\beta\) C\(_2\) g cc and \(\alpha\) C\(_2\) g cc conformers may add the possibility that the sweetener interacts with the receptor through more places, altering its relative sweetness.

### Conclusions

The conformational behaviour of ketohexoses was investigated for the first time by using a combination of Fourier transform microwave spectroscopy and laser ablation. In the isolation conditions of the gas phase, three, two and one rotamers were detected for \(\alpha\)-tagatose, \(\alpha\)-psicose and \(\alpha\)-sorbose, respectively. All are found to be over-stabilised by cooperative networks of intramolecular hydrogen bonds between vicinal hydroxyl groups stretching throughout the whole molecule.

The detailed structural information extracted from our experiments provides information on the orientation of the OH groups with respect to the molecular frame, and allows the intramolecular interactions in which these functional groups are involved to be established. Hence, a singular structural signature involving the hydroxyl groups \(\text{OH}_\text{gest}\) and \(\text{OH}_\text{gest}\) in the short H-bond network \(\text{OH}_\text{gest} \cdots \text{OH}_\text{gest} \cdots \text{O}_\text{ring}\) has been characterised for the first time in the most abundant species of ketohexoses indicating that anchoring to the sweet receptor occurs here, in accordance with Shallenberger’s old proposal.

In addition, a hydrophobic \(\gamma\) site surrounding the \(\text{C}_\text{gest}\) has been identified in all conformers, supporting the AH-B-\(\gamma\) “sweetness triangle” of Kier’s proposal. The high resolution reached by our LA-CP-FTMW experiments has opened a new window to explore the linkage between sweetness and structure.

Further investigation exploring the influence of water in conformational behaviour is being undertaken. In vacuum, the formations of Figure 10 show common surroundings of C\(_{10}\) free of any hydrophilic group. This site constitutes the main hydrophobic environment and, consequently, is the most suitable for being considered as \(\gamma\) in accordance with the aforementioned sweetness theory. The degree of sweetness of ketohexoses can also be considered in light of the present results. It is well known that \(\alpha\)-fructose is twice as sweet as \(\alpha\)-sorbose. Surprisingly, the conformational behaviour observed for both sugars, shown in Figure 10a and d, revealed extraordinary similarities. Hence, the most abundant species of \(\alpha\)-fructose and \(\alpha\)-sorbose are stabilised by the same H-bond network \(\text{OH}_\text{gest} \cdots \text{OH}_\text{gest} \cdots \text{OH}_\text{gest} \cdots \text{O}_\text{ring}\) spread over the entire molecule. Some authors have rationalised this difference in sweetness in terms of the existence of an intramolecular H-bond between the \(\text{OH}_\text{gest}\) and \(\text{O}_\text{ring}\) in \(\beta\)-fructopyranose and its absence in \(\alpha\)-L-sorbo.pyranose. However, no \(\text{OH}_\text{gest} \cdots \text{O}_\text{ring}\) hydrogen bond interactions are observed in any of the observed conformers of the ketohexoses. Speculating with the multipoint attachment theory, which also assume the presence of AH, B and \(\gamma\) sites in the glucophore, the distinct axial/equatorial orientation of the \(\text{OH}_\text{gest}\) in the \(\beta\) C\(_2\) g cc and \(\alpha\) C\(_2\) g cc conformers may add the possibility that the sweetener interacts with the receptor through more places, altering its relative sweetness.

### Conclusions

The conformational behaviour of ketohexoses was investigated for the first time by using a combination of Fourier transform microwave spectroscopy and laser ablation. In the isolation conditions of the gas phase, three, two and one rotamers were detected for \(\alpha\)-tagatose, \(\alpha\)-psicose and \(\alpha\)-sorbose, respectively. All are found to be over-stabilised by cooperative networks of intramolecular hydrogen bonds between vicinal hydroxyl groups stretching throughout the whole molecule.

The detailed structural information extracted from our experiments provides information on the orientation of the OH groups with respect to the molecular frame, and allows the intramolecular interactions in which these functional groups are involved to be established. Hence, a singular structural signature involving the hydroxyl groups \(\text{OH}_\text{gest}\) and \(\text{OH}_\text{gest}\) in the short H-bond network \(\text{OH}_\text{gest} \cdots \text{OH}_\text{gest} \cdots \text{O}_\text{ring}\) has been characterised for the first time in the most abundant species of ketohexoses indicating that anchoring to the sweet receptor occurs here, in accordance with Shallenberger’s old proposal.

In addition, a hydrophobic \(\gamma\) site surrounding the \(\text{C}_\text{gest}\) has been identified in all conformers, supporting the AH-B-\(\gamma\) “sweetness triangle” of Kier’s proposal. The high resolution reached by our LA-CP-FTMW experiments has opened a new window to explore the linkage between sweetness and structure.

Further investigation exploring the influence of water in conformational behaviour is being undertaken. In vacuum, the
isolated molecules can only make strained hydrogen bonds between adjacent hydroxyl groups around the edge of the ring. Incorporating environmental contributions such as solute–solvent interactions in aqueous solutions, which have the same nature as glucophore–receptor interactions,[48] should contribute to reinforcing our understanding of the structural features involved in sweetness.

**Experimental Section**

**LA-CP-FTMW spectroscopy**

Commercial samples of β-tagatose, α-psicose and α-sorbose (m.p.: 134 °C, 105 °C and 165 °C, respectively) were used without any further purification. Solid rods were prepared by pressing the compounds’ fine powder mixed with a small amount of commercial binder and were placed in the ablation nozzle (Figure S1 in the Supporting Information). A picosecond Nd:YAG laser (10 mJ per pulse, 20 ps pulse width) was used as a vapourisation tool. Products of the laser ablation (LA) were supersonically expanded by using the flow of carrier gas (Ne, 15 bar) and were characterised by chirped pulse Fourier transform microwave (CP-FTMW) spectroscopy. A Scheme of our LA-CP-FTMW spectrometer[25, 27] constructed at the University of Valladolid is shown in Figure S1a. A chirped-pulse is directly generated by an arbitrary waveform generator (1) and amplified by the adjustable (3) travelling wave tube (2) amplifier with 300 W maximum output power. Following amplification, a parabolic reflector system comprising dual ridge horns (5–6) and two parabolic reflectors (6–7) in a paraxial beam configuration is used to broadcast the excitation pulse into the vacuum chamber and receive the broad-band molecular emission. This molecular-free induction decay (FID) signal is directly digitised by using a digital oscilloscope (8) after it has been amplified with a low-noise microwave amplifier (9). The rotational spectrum in the frequency domain is obtained by taking a fast Fourier transformation of the FID, following the application of a Kaiser–Bessel window to improve baseline resolution. All frequency and trigger sources as well as the digital oscilloscope are phase-locked to a 10 MHz Rb-disciplined quartz oscillator (11).

The spectrometer operating sequence (Figure S1b) commences with a molecular pulse of 700 μs duration which drives the carrier gas flow through the pulsed valve source (I). After a slight delay, a laser pulse hits the solid and vaporizes the sample (II). Four separate broadband rotational spectra are acquired in each injection cycle (III). The four individual broadband chirped excitation pulses, of 4 μs duration, are spaced by 18 μs. Two μs after each excitation pulse ceases, the rotational free induction decay is then acquired for 10 μs (IV). Around 100 000 individual FIDs (four per each molecular expansion) at a 2 Hz repetition rate were averaged in the time domain and Fourier transformed to obtain the rotational spectra of β-tagatose, α-psicose and α-sorbose in the 6 to 12 frequency range.

**Computational methods**

Similar to other monosaccharides in condensed phase, ketohexoses present a six-member ring pyranose configuration. The carbon C1 becomes an asymmetric centre, which results in two possible α and β anomeric species. In aqueous solution, they mainly exist as a mixture of α and β pyranoses,[49] adopting the energetically favoured C1C and C1C chair conformations in which the hydroxymethyl group is in equatorial orientation (Figure 3). In addition, conformational analysis of ketohexoses entails considering intramolecular hydrogen bonding between OH groups taking place for each configuration. Plausible formation of hydrogen-bond networks reinforces the stability of certain conformers due to hydrogen bond cooperativity.[12] Knowledge of the subtle variation in hydroxyl arrangement is relevant to distinguish between different conformers and plays an important role in glucophore characterisation.

Identifying the different conformers present in supersonic expansion involves a combination of the aforementioned experimental approach and theoretical calculations. A typical assignment strategy, similar to that previously described in our studies of amino acids,[15] commences with the generation of the set of plausible conformers by performance extensive semi-empirical calculations (AM1 and PM3) to find the lowest-energy conformers in the potential energy surface. Those lower-energy conformers, which might conceivably be populated under experimental conditions employed in the supersonic expansion, are subsequently optimised by using the Møller–Plesset second order perturbation (MP2) theory to include electron correlation and larger 6–311G(d,p) basis set.[50] This level of theory has been found to behave satisfactorily in previous studies of sugars. These provide the relative energy of conformers, the rotational constants (A, B, C) and electric dipole moment components (μx, μy, μz) relevant for our rotational studies. Each conformer has distinct values for these spectroscopic parameters, which means they will give rise to different rotational spectra, enabling us to discriminate between them. Conformational identification can be achieved by comparing experimentally and theoretically predicted values of the above molecular properties, which collectively leads to identifying the observed conformers.

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