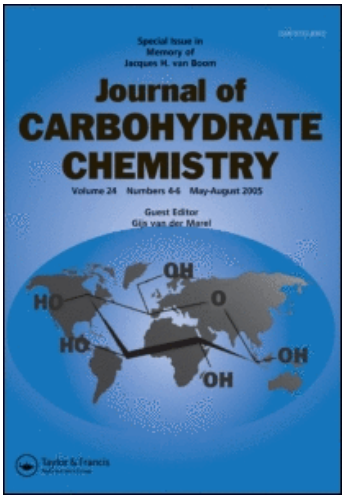

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Conformational Analysis of *C*-Trehaloses Using Molecular Mechanics Calculations

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ABSTRACT

Relaxed-residue energy maps based on the MM3 force field were computed for the three *C*-linked (1-1) D-glucosyl disaccharides, *C*-trehaloses: the axial–axial linked α,α-trehalose, the axial–equatorial α,β-trehalose and the equatorial–equatorial linked β,β-trehalose. Optimized structures were calculated on a 20°-grid spacing of the torsional angles about the *C*-glycosidic bonds. Boltzman weighted ^{3}J coupling constants were calculated and compared to the experimental values; they are satisfactory. The general shape of the energy maps indicates that α,α-trehalose is a quite rigid molecule adopting only one conformation around the *C*-glycosidic linkage, whereas the other two isomers are rather flexible. Compared to the corresponding *O*-disaccharides α,β- and β,β-trehaloses exhibit a larger number of low energy conformers and a larger area

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of the map energy < 8 kcal/mol. The preferred conformations of the axial *C*-glycosidic bond are in agreement with the *exo*-anomeric effect. Equatorial *C*- glycosidic bonds are rather flexible, influenced by the polarity of the milieu and the formation of interresidue hydrogen bonds.

Key Words: C-Glycosides; Trehaloses; Conformational analysis; Molecular modeling.

INTRODUCTION

Disaccharide analogues in which the glycosidic oxygen atoms are replaced by a methylene group have been synthesized as carbohydrate mimics as well as potential enzyme inhibitors.[1] The availability of such molecules provides the opportunity for investigating the influence of steric interactions around glycosidic bonds and evaluating the contribution of the *exo*-anomeric effect on the conformational preference at the glycosidic linkage.[2–12] Thus the detailed information on the conformational behavior in comparison with the *O*-analogues is of interest and has been discused in a recent review.[8] Early studies by Kishi and coworkers[2–4] postulated that *O*- and *C*-oligosaccharides adopt similar conformations; their conclusions were derived from a semi-quantitative conformational analysis based on a diamond lattice analysis of the steric effects and the NMR derived coupling constants. Moreover, they demonstrated that the bound conformation of *C*-lactose to peanut agglutinin is identical to that of its parent *O*-lactose bound to this lectin.[7] However, Jimenez-Barbero and coworkers proved that the active conformation of *C*-lactoside bound to ricin and β-galactosidase differs from that of the natural compound.[9,10] Conformational differences between *O*- and *C*-glycosides have been demonstrated also in the case of *C*-/*O*-mannobiose (α(1 → 2) linkage) and *C*-/*O*-α-Man(1 → 1)-β-Gal.[11,12] Recently, we have reported[13] a theoretical conformational analysis concerning eight *C*-linked D-glucosyl disaccharides ((1 → 2), (1 → 3), (1 → 4) and (1 → 6) linked D-glucopyranoses). It was concluded that although the shapes of the *C*- and *O*-disaccharide maps were similar, differences could be noted in the exact location, the relative energy and the number of the minima.

C-Trehaloses (Figure 1), the 1 → 1 linked D-glucopyranosyl, non-reducing disaccharides, are three isomers which differ only in the linkage configuration; α,α-trehalose (1) has two axial anomeric bonds, α,β-trehalose (2) has an axial and an equatorial and β,β-trehalose (3) two equatorial bonds.

The corresponding α,α-trehalose is widely distributed in nature[14] while the other two *O*-linked isomers are synthetic compounds. The analysis of their structures has

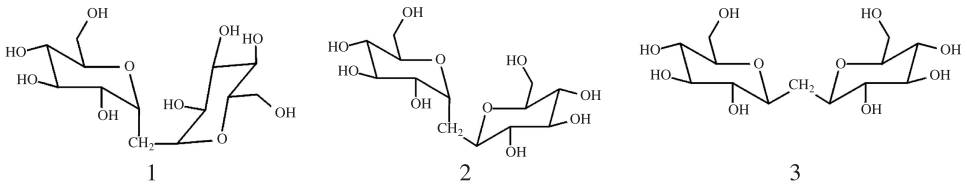


Figure 1. Schematic representation of the three *C*-disaccharides.

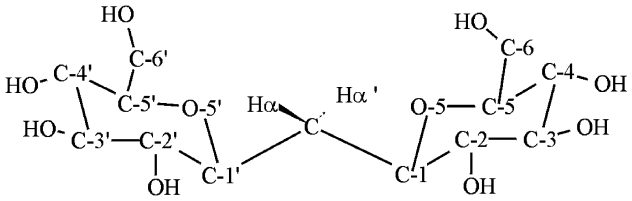
attracted some attention in order to investigate the differences between axial and equatorial glycosidic linkages,[15] and the magnitude of solvent effects, using Molecular Mechanics and Quantum Mechanics calculations.[16,17]

Kishi and coworkers[18] have synthesized the three *C*-trehaloses; they concluded from the NMR coupling constants and a qualitative analysis that the equatorial glycosidic bond deviates from the *exo*-anomeric effect only in the case of α,β-trehalose (axial–equatorial linkage). From their analysis it is not possible to clarify whether deviation means ''a conformer somewhat distorted from the *exo*-anomeric position'' or ''a mixture of staggered conformers.'' Jimenez-Barbero and coworkers investigated the conformational differences between α-*O*-Man-(1 → 1)-β-Gal and the *C*-analog. They concluded that the population distributions around the glycosidic linkages are rather different. This is particularly true for the α-*O*-Man linkage[12] where the non-*exo*-anomeric conformer was significantly populated (definition of the *exo*-anomeric effect is given in Refs. [2–7]). In the present study, relaxed-residue energy maps based on the MM3 force field have been established to study the conformational characteristics of *C*-trehaloses. Boltzmann-weighted ^{3}J coupling constants have been calculated and compared to the experimental values.[18] Comparisons are made between the modeling results for *C*- and *O*-trehaloses[15] and to other *C*-disaccharides.[13]

METHODS

Labeling of the atoms follows the IUPAC Nomenclature for Carbohydrates. The carbon atom at the *C*-glycosidic bond is labeled Cα, whereas the two hydrogen atoms are labeled Hα and Hα′ (Scheme 1). The torsional angles around *C*-glycosidic linkages are defined as following: Φ = O5′–C1′–Cα–C1, Φ′ = C1′–Cα–C1–O5. The three staggered orientations of the glucopyranosyl primary hydroxyl group are referred to as either *gauche–gauche* (*gg*), or *gauch–trans* (*gt*) considering the O5–C5–C6–O5 dihedral angle first and the C4–C5–C6–O6 second.

The Φ/Φ′ adiabatic maps of *C*-trehaloses were generated, as described previously,[19] using the MM3(92) force-field.[20–22] The relative orientation of the monosaccharide residues was varied in 20° increments over its full angular range, starting from forty-eight structures in the case of the α,β-trehalose. These structures were generated taking into account the *gg*, and *gt* conformations of the hydroxymethyl groups of each residue yielding four combinations, the orientation of the secondary hydroxyl group as *c* (clockwise) or *r* (anticlockwise) resulting in four combinations *cc*, *cr*, *rc*, *rr*, and three conformations for the C5–C6–O6–HO6 tortional angle (180°, 60°,



Scheme 1.

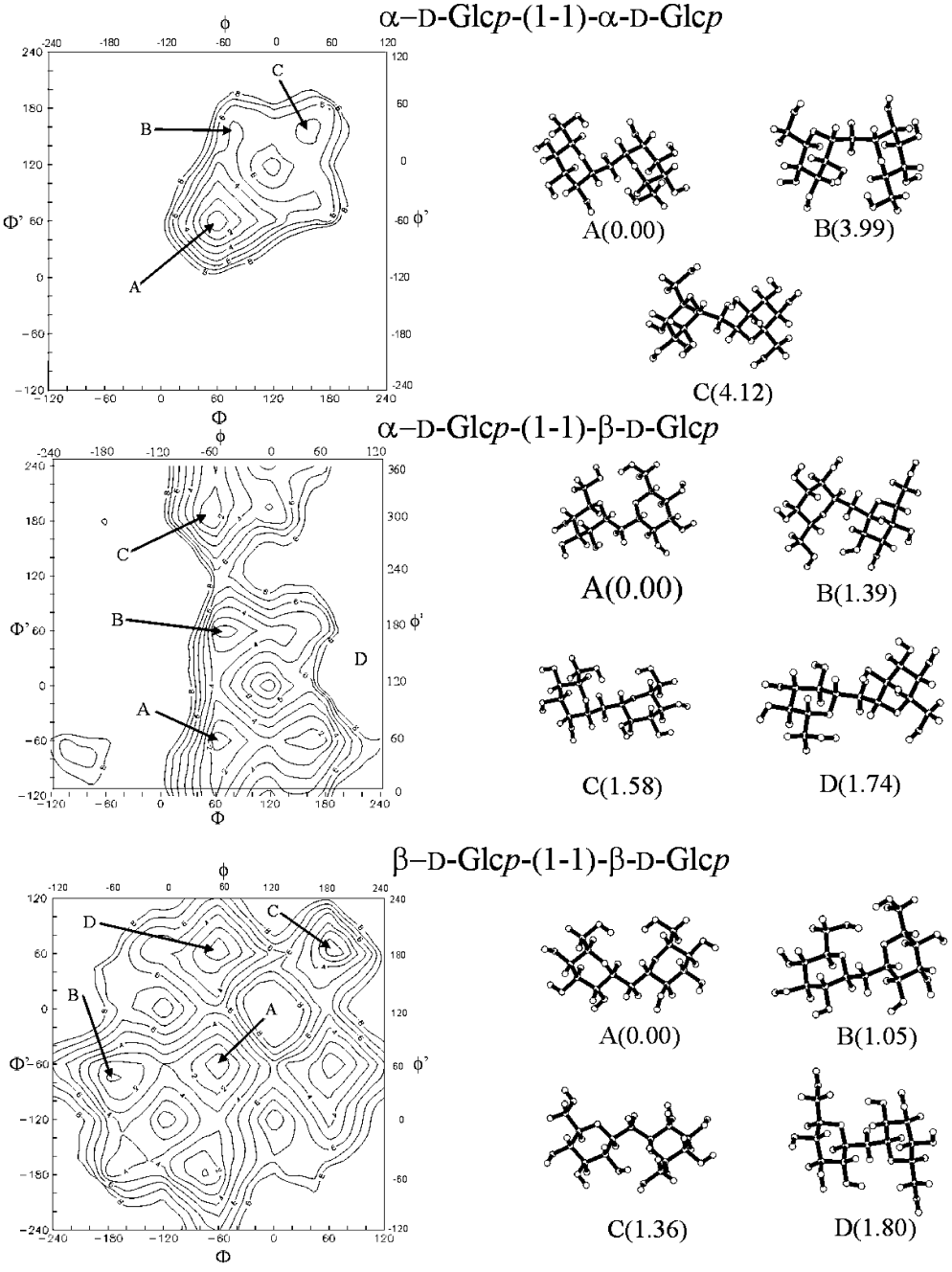


Figure 2. Adiabatic relaxed map of *C*-trehaloses in the MM3(92) force field as a function of the Φ and Φ′ glycosidic torsion angles. Isoenergy contours are drawn in 1 kcal/mol intervals out to the 8 kcal/mol from the global minimum. The lowest energy conformation of each domain has been represented by a ball and stick model, and its location is indicated on the map. Φ and Φ′ stand for the dihedral angles H1′–C1′–Cα–C1 and H1–C1–Cα–C1′, respectively.

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−60°). In the reverse-clockwise orientation, the secondary hydroxyl groups were positioned in such a way that OH3 was *anti* to C4, OH2 was *anti* to C3 etc., forming a crown of hydrogen bonds around the glucose ring. In the clockwise orientation, OH2 was positioned *anti* with respect to C1, OH3 *anti* with respect to C2 etc., forming the alternative crown. The *tg* position was not considered as it is not found in known structures of crystals.[15] In the case of the other two α,α-trehalose and β,β-trehalose only thirty starting structures were used since some combinations like *ggggcr* and *ggggrc* or *gggtcc* and *gtggcc* are identical because of the symmetry of the molecule. Starting from a refined geometry, the procedure drives Φ and Φ′ torsion angles in steps of 20° over the whole angular range while the MM3 program provides full geometry relaxation. The block diagonal minimization method, with the default energy-convergence criterion (0.00008*N kcal/mol per five iteration, where N was the number of atoms in the molecules). The bulk dielectric constant (ε) was set to 4.0 as this intermediate value models the crystalline glucopyranosyl ring better than either ε = 1.5 (vacuum) or ε = 80° (water). All calculations were performed on a Silicon Graphics O2 (R5000) computer. From these relaxed energy maps, adiabatic surfaces were built by selecting the lowest energy structure for a given Φ,Φ′. For each local low-energy region of a map, the conformation that contributed the lowest energy grid-point was submitted to a further, non-restricted full matrix minimization in order to find the local minimum. If the structure remained within the local well, the lowest energy structure was taken as the local minimum structure. Usually, the final location of the minima was found within the local regions, although they could have torsional angles several degrees removed from those of the grid-point structure.

Coupling constants, $^3J_{H,H}$ for vicinal hydrogen atoms of a H−C−C−H segment were calculated using the appropriate Altona equation.[23] $^3J_{H1H\alpha} = 4.781\cos(2\theta) - 0.990\cos(\theta) - 0.950\sin(2\theta) + 5.912$ and $^3J_{H1H\alpha'} = 4.781\cos(2\theta) - 0.990\cos(\theta) - 1.157\sin(2\theta) + 5.912$ for the axial bond and $^3J_{H1H\alpha} = 4.781\cos(2\theta) - 0.990\cos(\theta) + 1.157\sin(2\theta) + 5.912$ and $^3J_{H1H\alpha'} = 4.781\cos(2\theta) - 0.990\cos(\theta) + 0.950\sin(2\theta) + 5.912$ for the equatorial bond. Assuming that the entropy differences among the different conformers are negligible, the probability P_i of the *i*th conformer depends upon its relative energy $P_i = \exp(-E_i/RT)/\sum(\exp(-E_i/RT))$. Ensemble averages were calculated from the distribution according to $\langle {}^3J\rangle = \sum P_i J\theta$, Although in α,α- and β,β-trehalose the H_α and $H_{\alpha'}$ are equivalent they were technically differentiated in order to calculate the ^3J couplings as the experimental data concern unsymmetrically protected derivatives and the corresponding coupling constants were thus available.

CONFORMATIONAL ANALYSIS

The MM3-calculated surface energy for *C*-α,α-trehalose is shown in Figure 2 along with the location and the representation of the low energy conformers. The conformational features of the minima are listed in Table 1. The adiabatic map of this axial−axial isomer comprises a single well. The lowest energy conformer A is located on a perfectly staggered conformation for both Φ = 60° and Φ′ = 60°. Two other minima at Φ/Φ′ = 79.6°/160.7° and Φ/Φ′ = 160.7°/79.6° correspond to the same structure B, as the map is symmetrical with respect to its diagonal. A third low energy structure is located at Φ/Φ′ = 159°/159°.

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Table 1. Energy minima for MM3-generated relaxed-residue analysis of disaccharides. Comparison with correspondent *O*-glycosides.

		C-glycosides				*O*-glycosides[a]			
		Location of minimum				Location of minimum			
Disaccharide	Conformer	Φ	Φ′	Energy	Rel. energy	Φ	Φ′	Energy	Rel. energy
AA	A	59.9	59.9	34.01	0.00	75.5	75.5	18.68	0.00
	B	160.7	79.5	38.00	3.99	90.9	145.3	23.34	4.66
	C	158.8	158.9	38.13	4.12	145.3	90.9	23.34	4.66
AB	A	68.1	− 65.2	31.22	0.00	78.4	−70.1	18.21	0.00
	B	77.1	62.2	32.61	1.39	93.7	57.3	21.54	3.33
	C	55.4	− 176.5	32.80	1.58	−81.3	−81.0	26.51	8.30
	D	159.9	− 60.6	32.96	1.74				
BB	A	− 59.8	− 60.9	28.16	0.00	−76.2	−76.2	16.97	0.00
	B	− 61.2	62.4	29.21	1.05	−63.7	62.7	20.90	3.93
	C	− 80.5	− 178.1	29.52	1.36	62.7	−63.7	20.90	3.93
	D	72.1	67.6	29.96	1.80	70.9	70.9	23.43	6.46

[a]From Ref. [15].

Table 2. Starting structures contributing to MM3-generated relaxed-residue Φ,Φ′ disaccharide maps.

	Complete map					Lowest 8 kcal/mol			
Disaccharide	Hydroxy-methyl		Secondary hydroxyls		% Area of map	Hydroxy-methyl		Secondary hydroxyls	
AA	*gggg*	36.4%	*cc*	38.0%	24.4%	*gggg*	11.4%	*cc*	11.4%
	gggt	4.9%	*cr*	25.9%		*gggt*	0.0%	*cr*	25.6%
	gtgt	58.6%	*rc*	0.3%		*gtgt*	88.6%	*rc*	0.0%
			rr	35.8%				*rr*	62.0%
AB	*gggg*	0.9%	*cc*	4.9%	43.2%	*gggg*	0.8%	*cc*	2.9%
	gggt	4.6%	*cr*	23.2%		*gggt*	2.1%	*cr*	13.6%
	gtgg	5.9%	*rc*	46.9%		*gtgg*	2.1%	*rc*	50.7%
	gtgt	88.6%	*rr*	25.0%		*gtgt*	95.0%	*rr*	32.8%
BB	*gggg*	1.5%	*cc*	19.8%	66.4%	*gggg*	1.9%	*cc*	16.3%
	gggt	3.7%	*cr*	39.2%		*gggt*	2.3%	*cr*	43.7%
	gtgt	94.8%	*rc*	0.3%		*gtgt*	95.8%	*rc*	0.5%
			rr	40.7%				*rr*	39.5%

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None of the minima are stabilized by the formation of any inter-residue hydrogen bond. According to our calculations, the relative energies of minima B and C are > 4.2 kcal/mol greater than the lowest energy and it is unlikely that these minima exist in any significant extent in solution.

This suggests that structure A is the only adopted conformation. The *gtgt* initial orientation of the hydroxymethyl groups covers 89% of the low energy conformers whereas *rr* is the orientation of the secondary hydroxyl groups is ~62% (Table 2).

The MM3 generated map of α,α-trehalose reported previously,[15] using the same dielectric constant, shows similar characteristics. Practically only one conformer exists for the *O*-disaccharide in a conformation that is shifted from the staggered position for both Φ and Φ′ for 20° showing the importance of the *exo*-anomeric effect. *O*-α,α-trehalose seems to be more rigid as only 14.2% of the conformational space has relative energy lower than 8 kcal/mol while the low energy conformational space in the *C*-disaccharide map is 24.4% (Table 2).

The ^{3}J coupling constants between protons Hα, Hα′, H1 and H1′ were calculated (Table 3) and compared with the experimental values reported by Kishi[18] for an unsymmetrically protected derivative of α,α-*C*-trehalose. A quite satisfactory agreement between calculated and measured values is reached (Scheme 2). The experimental value $J_{H1'H\alpha'} = J_{H1H\alpha} = 3.5$ Hz is 1.4 Hz larger than the computed one, which could indicate the occurrence of B and C conformers in solution. In the case of the natural compound, the occurrence of the B conformer is still a matter of a controversy. It is the global minimum found from application of the CHARMM force field[17] in contradiction to the results of the other molecular mechanics calculations using MM3[15] or more recent QM calculations.[16]

For both residues the ''glycosidic bond'' conforms to the *exo*-(*syn*)-anomeric effect. Other axial–axial linked disacharides like *C*-mannobiose showed that the ''glycosidic'' bond could adopt conformations like structure C, to a significant extent challenging the rigidity about axially linked bonds.[11]

The adiabatic map of *C*-α,β-trehalose is shown in Figure 2. Four different minima can be observed and their geometric characteristics are summarized in Table 1. Three of the four lowest energy conformers A, B and C are located in a trough along the Φ′ direction with relative energies less than 2 kcal/mol. Conformer A (Φ/Φ′ = 68.1°/ − 65.2°), is the global minimum in this map and has an energy 1.4 kcal/mol lower than that of the B conformer. Its conformation is stabilized by the occurrence of an inter-residue hydrogen bond between O6′...O6. As for the B conformer, an inter-residue hydrogen bond between O6′ and O2 is found. None of the other low energy structures exhibit an inter-residue hydrogen bond. In conformer A both equatorial and axial ''glycosidic'' bonds are consistent with the *exo*-(*syn*)-anomeric effect. As for the B conformer, the conformation of the equatorial bond places C1′ *gauche* to both O5 and C2 (*exo*–*anti*). Conformers C and D have relative energies of about 2 kcal/mol over the lowest energy minimum; it may be concluded that the relative flexibility along the Φ′ axis suggests that the axial ''glycosidic bond'' is more rigid compared to the equatorial bond.

The calculated ^{3}J couplings between protons Hα, Hα′, H1 and H1′ are summarized in Table 3. Some deviations are observed between calculated and experimental values[18] (Scheme 3). Whereas the coupling constants about the axial bond are well predicted, the one corresponding to the equatorial bond, $J_{H1H\alpha}$ is not in agreement with the experimental values.

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Table 3. Calculated vicinal coupling constants across the C1′–Cα–C1 bridge of trehaloses.

Disaccharide	Coupling constants			
	$J_{H1'H\alpha}$	$J_{H1'H\alpha'}$	$J_{H1H\alpha}$	$J_{H1H\alpha'}$
α,α-*C*-trehalose	11.5	2.1	2.1	11.4
β,β-*C*-trehalose	2.7	9.9	10.0	2.8
α,β-*C*-trehalose	10.0	3.0	9.3	3.6

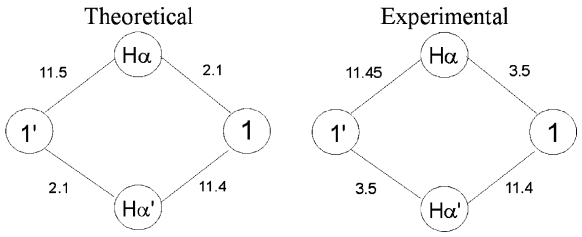
Not surprisingly, it has to be noted that the experimental data reported by Kishi show that there is a net dependence of ^3J couplings from the solvent. This effect is more important in the $J_{H1H\alpha}$ varying from 7.2 Hz in water to 5.5 Hz in pyridine exhibiting intermediate values in MeOH and DMSO.[18] Furthermore, when the OH groups are protected the solvent dependence is less marked.

These experimental observations, along with our theoretical analysis suggest that an equilibrium of staggered conformations and not a unique distorted rigid conformation[18] is likely to exist in solution. Conformers A and B seem to contribute mainly in the final value of the J couplings. A relative ratio between the two conformers in favor of B would result in an increase of the $J_{H1H\alpha}$ value. The population of the area around the A conformer represents the 48% of the whole population at room temperature; whereas the populations of B, C and D are 3%, 6% and 4%, respectively.

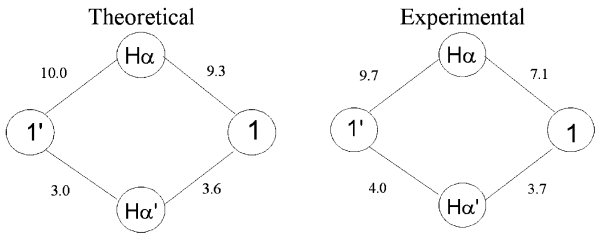
Moreover, this equilibrium should be influenced by the relative strength of the inter-residue hydrogen bonds formed in conformers A and B which depend on the polarity and the nature of the solvent.

When the adiabatic map was calculated using a lower dielectric constant ε = 1.5 conformer B became the global energy minimum exhibiting a slight difference of 0.03 kcal/mol with respect to conformer A, (conformers C and D having relative energy of 2.55 and 1.98 kcal/mol, respectively (data not shown)). The calculation of the coupling constants based on this map (Scheme 4A) indicates a good agreement for $J_{H1'H\alpha}$ whereas $J_{H1H\alpha}$ is still higher than the experimental. The relative populations of the regions around A, B, C and D represent the 51, 27, 0 and 1% respectively showing that conformer A is still predominant.

Instead, if we take into account only the four conformers and their relative energies to calculate the coupling constants, then the relative populations A/B/C/D are 47.5/50.0/0.6/1.6% and the coupling constant $J_{H1H\alpha}$ is well predicted (Scheme 4B). There is still a



Scheme 2.

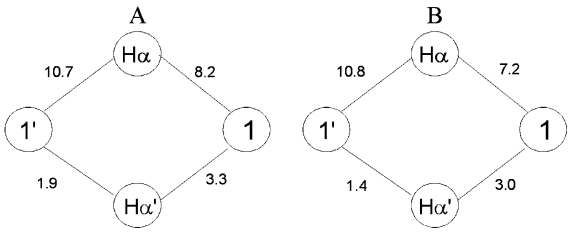


Scheme 3.

deviation between the calculated and observed coupling constant, $J_{H1'H\alpha'}$, suggesting that conformer D should also exist in solution with an occurrence of about 4%.

An equilibrium between the A, B and D conformers should exist in solution. This equilibrium is influenced by the strength of the inter-residue hydrogen bonds formatted in each conformer, which is also influenced by the solvent. According to that it seems less probable that there is a weakening of the *exo*-anomeric effect due to the solvent as was suggested.[18] The experimental data for the axial bond $J_{H1'H\alpha} = 9.7$ Hz and $J_{H1'H\alpha'} = 4.0$ deviate from the values 11.5 and 2.1 Hz that would be predicted for a conformation dictated by the *exo*-anomeric effect. This indicates the occurrence of contributions from conformer D, the result of which places C1 in an *anti* orientation with respect to O5′ (non-*exo* conformation). The occurrence of such a non-*exo* conformation for an axial ''glycosidic bond'' has also been suggested; it may be highly populated in solution as in the case of *C*-α-Man(1 → 1)-β-Gal.[12] The calculations suggest also that MM3 does not reproduce well the energy differences between the conformers probably due to an unsatisfactory calculation of the hydrogen bond energy and of course the limitation of calculations performed without taking into account explicit solvent molecules.

The corresponding adiabatic map of α,β-trehalose[15] differs from that of the *C*-analogue. There is only one low energy region parallel to Φ′ axis with only two minima corresponding to A and B. The two other low energy minima C and D of the *C*-analogue exist only as plateau regions in the trehalose map. The global minimum is the one corresponding to A with an energy difference of 3.3 kcal/mol from the next lower energy conformer, B. The energy barrier between the A and B conformers in *O*-trehalose is >8 kcal/mol and in *C*-trehalose <6 kcal/mol. A major difference between the two maps is that in the *O*-glycoside the conformational space with energies less than 8 kcal/mol is



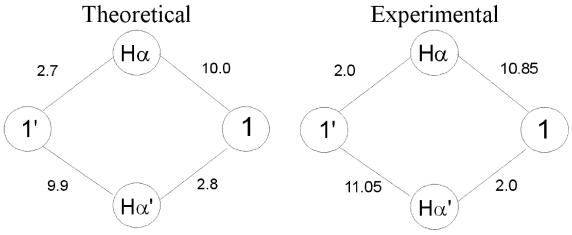
Scheme 4.

25% of the map area for the *O*-trehalose and 43.8 for the *C*-analogue. Such a difference is also reported for the rigid Φ/Φ′ map of the *N*-analogue of peracetylated α,β-trehalose where again the accessible area is larger but the difference is less important.[24] The crystal structure of the *N*-α,β-trehalose analogue corresponds to conformer A. However, because the secondary hydroxyl groups are protected, the formation of inter-residue hydrogen bonds is not possible. The crystal structure of *O*-α,β-trehalose corresponds to conformer A, with the primary hydroxyl orientations being *gt*−*gt*; this is accompanied by the occurrence of an inter-residue hydrogen bond between O6′ and O6.[25] In the crystal structure, a water molecule participates in the stabilization of the conformation showing the importance of the solvent molecules.

The Φ/Φ′ map of *C*-β,β-trehalose is shown in Figure 2 along with the representation of the low energy structures; the geometrical features of the conformers are listed in Table 1. This map is also typical of di-equatorially linked symmetric molecules, and as in the case of the *C*-α,α-trehalose map exhibits symmetry. It consists mainly of two perpendicular troughs intersecting in the region of the global minimum. Four low energy structures exist. The lowest energy structure A is located at a perfectly staggered position Φ/Φ′ = − 60°/ − 60°. This conformation is consistent with the *exo*-anomeric effect for both ''glycosidic'' bonds. Conformer B at Φ = − 60° and Φ′ = 60°, has a relative energy of 1 kcal/mol higher than conformer A; it could accommodate a weak hydrogen bond between OH6′...O5. In this conformer, the orientation of only one of the two glycosidic bonds is consistent with the *exo*-(*syn*)-anomeric effect. Conformer C (Φ/Φ′ = − 80°/180°) has a relative energy of 1.36 kcal/mol with respect to conformer A; it is stabilized by the formation of two hydrogen bonds between OH2′ and O5 as well as with O6. In this conformer one of the ''glycosidic'' bonds adopts a non-*exo* orientation. Conformer D at Φ = 60° and Φ′ = 60° could accommodate two symmetric hydrogen bonds between OH2...O5′ and OH2′...O5 stabilizing it at a relative energy of 1.80 kcal/mol.

The calculated ^{3}J coupling constants between protons H1, Hα, Hα′ and H1′ are listed in Table 3. The theoretical and experimental coupling constants are schematically described in Scheme 5. Compared to the experimental values reported by Kishi[18] for an unsymmetrically substituted analogue they are in quite good agreement.

The experimental J couplings of *C*-β,β-trehalose are somehow in contradiction with the corresponding J couplings measured for *C*-α,β-trehalose.[18] The flexibility around the equatorial ''glycosidic'' bond, observed in the *C*-α,β-trehalose is not observed in the di-equatorially linked analog. This difference should be attributed to the fact that the J couplings measured for the *C*-β,β-trehalose concern a unsymmetrically



Scheme 5.

protected derivative while the J couplings measured for the *C*-α,β-trehalose concern an unprotected molecule. This is in agreement with the MM3(96) conformational analysis, recently reported by French and coworkers, for *O*-α,α-trehalose and *O*-β,β-trehalose in their native and permethylated forms.[26] In their investigation the permethylated analogues were found to be less flexible than the native molecules. Moreover, the flexibility of the native structure of *O*-β,β-trehalose depends upon the dielectric constant used for the energy calculation. These results strongly support the analysis described above concerning *C*-α,β-trehalose. The same observations hold for *C*-β,β-trehalose. When the energy differences between the low energy structures were calculated with dielectric constant $\varepsilon = 1.5$ the global minimum was found to be conformer C, as this conformation is stabilized by the occurrence of the two hydrogen bonds between OH2′ and O5 as well as with O6. Thus, the resulting decrease in *exo*-anomeric stabilization is a result of the increasing strength of the hydrogen bond.

Compared to the MM3 generated map of the β,β-trehalose,[15] the map of the *C*-analogue exhibits again substantial differences. The global minimum in *O*-trehalose is located in approximately the same position but it is shifted from the staggered conformation. Moreover, it exhibits larger energy difference, i.e., >4 kcal/mol, with all other conformers. The energy barriers between the low energy conformers are lower for *C*-trehalose (<5 kcal/mol) than for the *O*-analogue (>8 kcal/mol). As in the case of α,β-trehalose, the region of the conformational space that is limited by the 8 kcal/mol barrier is considerably larger (66.4%) than in the case of *O*-disaccharide (35%).

The differences between *C*- and *O*-MM3 maps are similar with those observed in other D-glucosyl disaccharides studied previously; they exist already in the MM3 energy profile of the glycosidic bond of ethyl glycopyranoside and the $-CH_2-$ linked analogue.[13]

The comparison between the three *C*-trehalose potential energy surfaces indicates that the equatorial *C*-''glycosidic'' bonds are much more flexible than the axial ones, and that an equilibrium of conformers exists in solution, as it has been previously postulated in the case of natural counterparts. The comparison with other *C*-disaccharides indicates that the area of low energy conformations (<8 kcal/mol with respect to the global minimum) is larger for α,β-trehalose than for the other axially–equatorially linked *C*-disacharides as *C*-kojibiose (37.6%), *C*-nigerose (38.9%) and *C*-maltose (31.2%).[9–12] The same holds true for β,β-trehalose in comparison with other equatorially–equatorially linked *C*-disaccharides as *C*-sophorose (62.0%), *C*-laminarabiose (59.9%) and *C*-cellobiose (55.5%). This suggests that the $1 \rightarrow 1$ linkage is more flexible than $1 \rightarrow 2$, $1 \rightarrow 3$ or $1 \rightarrow 4$ and it is not in agreement with the suggested trend in conformational rigidity ''equatorial *C*-glycosidic bond > *C*-aglyconic bond''.[18]

These differences were not observed in the corresponding *O*-disaccharide calculated maps.[15,27,28] For instance, in the corresponding of *O*-laminarabiose, 35.2% of the map has energy values within 8 kcal/mol of the global minimum, in β,β-trehalose the corresponding percentage is almost indentical: 34.9%. Moreover, this percentage is considerably higher for all *C*-diglycosides compared to their *O*-counterparts. In *C*-disaccharides it depends on the type of linkage, and it decreases as the bulk of the substituents near the glycosidic bond increases. These results show that steric effects control the conformational stability in *C*-glycosides. In the corresponding *O*-disaccharides the stereoelectronic nature of the *exo*-anomeric effect imposes a more rigid structures where the orientation of C1 is mainly *gauche* with respect to O5′.

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In conclusion the above MM3 based conformational analysis implies that in *C*-trehaloses the *C*-''glycosidic'' bonds adopt staggered conformations. *C*-trehaloses seems to be more flexible than other *C*-diglycopyranosides. In conformity with the experimental results our theoretical calculations support that the axial *C*-''glycosidic'' bonds are relatively rigid and consistent with the stabilizing infuence of the *exo*-anomeric effect. The equatorial *C*-''glycosidic'' bonds are rather flexible adopting different staggered conformations in an equilibrium that is driven by the occurrence of inter-residue hydrogen bonds.

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