Solution Conformations of β,β-Trehalose and Its *C*-Disaccharide Analog from Optical Rotation

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We report evidence that β,β-trehalose[1a] (Figure 1a) and its *C*-disaccharide analog[1b] (Figure 1b) adopt similar "zigzag"[2] conformations in aqueous solution, the implication being that the *exo*-anomeric effect, necessarily absent in the latter, cannot be presumed to be conformation determining in the former. In addition to their role as model compounds for elaborating conformational determinants in saccharides, *C*-disaccharides are of interest as potential active-site-directed inhibitors of glycosidases and, thereby, as pharmacological regulators of glycoprotein processing and cell surface expression.[3]

The NMR equivalence of residues in β,β-trehalose precludes determination of its solution conformation through conventional NMR techniques involving the measurement of chemical shifts, relaxation times, coupling constants, or NOEs. Attempts to make the two glucose rings distinguishable by NMR, as through isotopic substitution, have not yet been reported. Martin and Lai,[4] by exploiting the magnetic nonequivalence of the methylene bridge protons in *C*-β,β-trehalose (H7, H7′), simulated their NMR signal to determine the extent of coupling with H1 and H1′. Values of $J_{1,7} = J_{1',7'} = 9.6$ Hz and $J_{1,7'} = J_{1',7} = 2.4$ Hz indicate unambiguously that each H7 proton is *anti* with respect to one H1 and *gauche* with respect to the other; a relatively inflexible linkage is evidenced by the nearly maximal value of 9.6 Hz.[4] Two linkage conformations[5] are compatible with the NMR results: approximately $\phi, \psi = 60°, 60°$ and $-60°, -60°$. Citing unfavorable steric interactions between O2 and H1′ in the latter conformation, the authors assigned the conformation to $\phi, \psi = 60°, 60°$. In that conformation, the C1–C2 bond of each glucose ring is *trans* to the C7–C1 bond of the other, and the C7–C1 bond of each ring is *gauche* to the C1–O5 bond of the other; the result is an extended zigzag arrangement[2] of seven carbon atoms, C3 through C3′.

MM3 flexible-residue vacuum calculations[6] on the parent disaccharide, β,β-trehalose, indicate a global energy minimum at $\phi, \psi = 44°, 44°$. The next lowest energy minimum is 3.9 kcal mol^{-1} higher, near $\phi, \psi = 60°, 180°$. The recently reported X-ray-determined solid-state conformation[7] is $\phi, \psi = 45°, 45°$ ($-76°$ for O5–C1–O1–C1′; 167° for C2–C1–O1–C1′). Therefore, in the absence of a direct NMR determination of the β,β-trehalose solution conformation, only indirect evidence exists for concluding that the two compounds have similar conformations in solution.

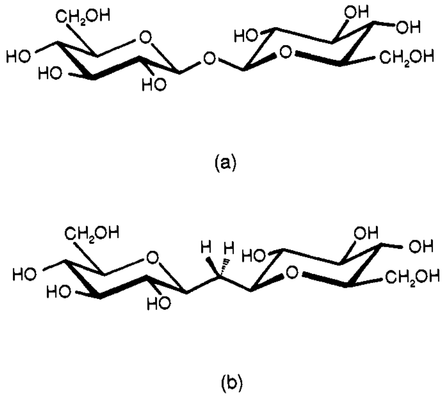

Figure 1. (a) β,β-Trehalose. (b) *C*-β,β-Trehalose.

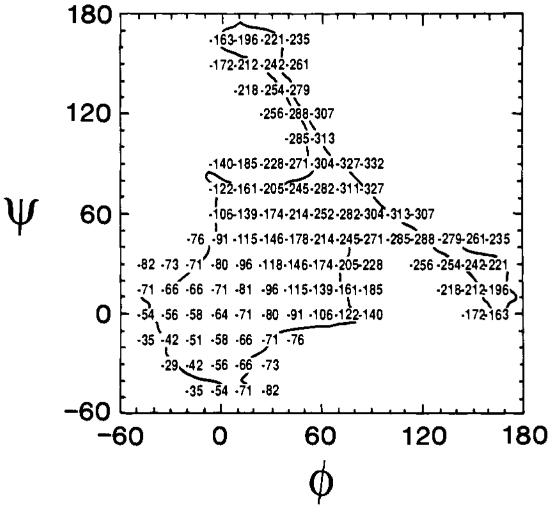

Figure 2. Molar rotations of β,β-trehalose calculated as a function of linkage angles ϕ and ψ, superimposed on a hard-sphere conformation map.

The molar rotations of the two compounds in solution are, however, significantly different: $[M]_D = -138$ deg cm^2 dmol^{-1} for β,β-trehalose[8] and $[M]_D = -69$ deg cm^2 dmol^{-1} for its C–analog.[4] We therefore applied a recently developed semiempirical model[9] of saccharide optical rotation to determine whether the difference in rotation is consistent with similar solution conformations. The model was previously applied to α,α-trehalose,[9b] in which case the calculational model accurately reproduced the observed optical rotation for the linkage conformation found as the global energy minimum in modeling calculations and in solid-state X-ray studies.

Atomic coordinates were adapted from those of Arnott and Scott.[10] The linkage valence angle was taken to be 117° in β,β-trehalose and 109.7° in the C-analog; the C1–C7 and C7–H7 bond lengths in the latter were taken to be 1.52 and 1.10 Å, respectively. *gt* and *gg* hydroxylmethyl group conformations were weighted equally.[11] The uncertainty in the model is estimated[9a] to be ±24 deg cm^2 dmol^{-1}. Results are displayed in Figures 2 and 3, superimposed on hard-sphere φ, ψ maps, generated using atom–atom contact distances of Rees and Scott.[12] If alternative

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[1] (a) β-D-Glucopyranosyl-(1→1)-β-D-glucopyranoside; isotrehalose. (b) *C*-β,β-Trehalose or bis(β-D-glucopyranosyl)methane.

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[5] In trehalose, ϕ and ψ are the dihedral angles H1–C1–O1–C1′ and C1–O1–C1′–H1′, respectively. In *C*-trehalose, ϕ = H1–C1–C7–C1′ and ψ = C1–C7–C1′–H1′.

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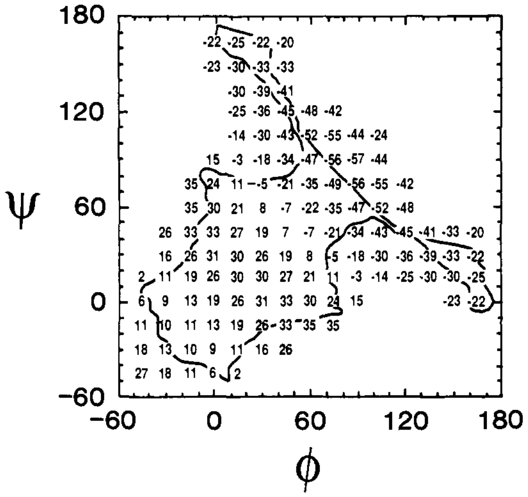

Figure 3. Molar rotations of *C*-β,β-trehalose calculated as a function of linkage angles ϕ and ψ, superimposed on a hard-sphere conformation map.

gt:*gg* statistical weights of 0.67:0.33 are used,[9a] the magnitudes of the optical rotations in Figures 2 and 3 are reduced by approximately 16% and 9%, respectively.

In β,β-trehalose (Figure 2), the MM3 minimum energy conformation (ϕ, ψ = 44°, 44°)[6] yields a calculated rotation of −178 deg cm^2 dmol^{-1}, which is within 1.4 standard deviations of the observed value (−138 deg cm^2 dmol^{-1}); with an 0.67 *gt* hydroxylmethyl statistical weight, the calculated value is −149 deg cm^2 dmol^{-1}. Rotations of −146 deg cm^2 dmol^{-1} are calculated for conformations which differ from the MM3 global energy minimum conformation by only 15° (φ, ψ = 30°, 45° = 45°, 30°); such conformations are within the 1 kcal mol^{-1} energy contour above the energy minimum.[6] The optical rotation of the next lowest energy MM3 conformation is −220 deg cm^2 dmol^{-1}. The present interpretation of optical rotation is thereby consistent with the MM3 potential energy surface. Both methods appear to indicate that there may be a slight departure from the idealized zigzag structure in solution, as well as in the solid state.

In *C*-β,β-trehalose (Figure 3), replacement of the linkage oxygen atom with a methylene group leads to less negative calculated rotations for all linkage conformations (compare Figure 2). Conformations within 15° of the idealized zigzag structure, e.g., φ, ψ = 75°, 75°, yield calculated rotations (−49 deg cm^2 dmol^{-1}) not significantly different from the observed value (−69 deg cm^2 dmol^{-1}). The φ, ψ = −60°, −60° linkage conformation, also compatible with the NMR data, is not compatible with the observed optical rotation. The apparent departure from the idealized zigzag structure may not be significant given the rigid residue geometries and linkage valence parameters used in the optical calculation. Therefore, to within ±15° in ϕ and ψ, the optical rotation is consistent with the NMR data.

The present optical rotation analysis leads to the following conclusions: (a) the NMR of *C*-β,β-trehalose[4] reflects the presence of a linkage conformation near ϕ, ψ = 60°, 60° and (b) for β,β-trehalose, the vacuum MM3 flexible-residue molecular modeling calculation,[6] indicating a deep energy minimum near ϕ, ψ = 44°, 44°, is consistent with the optical rotation observed in aqueous solution; the two methods together allow a conformational assignment, even in the absence of NMR data. Even with the uncertainties in all three methods (NMR, molecular modeling, and optical rotation), the conclusion remains that β,β-trehalose and *C*-β,β-trehalose adopt in aqueous solution conformations which are very similar.

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