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# STEREOCHEMISTRY OF NONREDUCING DISACCHARIDES IN SOLUTION*

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ABSTRACT

A theoretical, conformational analysis of three isomeric forms of nonreducing disaccharides in solution has been carried out using 2-(tetrahydropyran-2-yloxy)tetrahydropyran as a model. PCILO calculations of the two-dimensional ($\Phi$, $\Phi'$) maps showed that the rotation around the C–O bonds depends on the orientation of neighboring C–O bonds. The structure of every stable conformer was optimized by PCILO and MM2CARB methods. The abundance of conformers depends on the solvent, and this is also reflected in calculated average values of the carbon–proton constants and the linkage rotation. An exception is the $\alpha,\alpha$-form, in which the abundance of the favored conformer (96%) does not alter. The calculated structure and solution behavior of the $\alpha,\alpha$-form are in good agreement with the experimental data available. The energies estimated for 13 individual conformers made it possible to calculate the environmental dependence of the magnitude of the anomeric (0.5–4.0 kJ/mol) and exo-anomeric (1.5–10.1 kJ/mol) effects.

## INTRODUCTION

Trehalose is the general name given to a family of three D-glucosyl D-glucosides, nonreducing disaccharides which form the reserve carbohydrates of insects and a number of invertebrates, and are a storage material of fungi, yeast, and algae[1]. In bacteria, the precise serological differentiation, most of the overt antigenicity and features of the pathogenesis of many spacies are due to a family of trehalose-containing lipo-oligosaccharides[2].

Three linkages are possible, namely, $\alpha,\alpha$, $\alpha,\beta$, and $\beta,\beta$, and all three anomeric configurations of disaccharide with two pyranose rings are known, but, in general, only $\alpha,\alpha$-trehalose is the common isomer, although $\alpha,\beta$-trehalose has been isolated from honey[3]. The crystal structure of $\alpha,\alpha$-trehalose in the dihydrate[3,4] and the anhydrous[5] form has been determined. In both, the molecule has approximate

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$C_2$ symmetry, with the exception of the orientation of the primary alcohol groups, which are *gauche, gauche* on one residue and *gauche, trans* on the other. Two glycosidic-linkage torsion angles are more nearly equal in the anhydrous structure: 60.8 and 60.1°, *versus* 74.8 and 61.7° in the dihydrate[3,4].

The structure and conformation of trehalose in solution were studied by $^{13}C$- and $^{1}H$-n.m.r.-spectral measurements[6–8] Unusual upfield shifs for anomeric carbon atoms of $\alpha,\alpha$- and $\beta,\beta$-trehalose from the corresponding $\alpha,\beta$-trehalose were observed and interpreted as being a consequence of the exo-anomeric effect[6,7]. The n O.e. results observed for $\alpha,\alpha$-trehalose were in good agreement with a conformation having $\Phi$ values of $-50°$, as inferred from HSEA calculations[8]. Also, $\alpha$-D-glucopyranosyl $\alpha$-D-mannopyranoside showed $^{3}J_{COCH}$ values of 3 Hz for both anomeric carbon atoms[8], which indicates angles of about $-50°$. Therefore, the authors concluded that the weighted average, of the orientations around the glycosidic bonds in $\alpha,\alpha$-trehalose is populated near the $\Phi$ values of $-50°$.

We now report the results of theoretical conformational analysis of the three trehaloses. The incentive for determining the solution behavior of the trehaloses was to compare the orientational properties around a glycosidic linkage in the three different orientations of the C–O–C–O–C–O–C segment, and to aid understanding of anomeric and exo-anomeric effects

*The model and the method applied*

Conformational properties of the nonreducing disaccharides have been studied by using 2-(tetrahydropyran-2-yloxy)tetrahydropyran (DTHP) as a model; for numbering of the atoms, see Fig. 1. The rotation around the glycosidic linkage is given by the angles $\Phi = \Phi$ [O-5–C-1–O-1–C-1′] and $\Phi' = \Phi$ [O-5′–C-1′–O-1–C-1]. The orientational properties of the glycosidic linkage in the three isomeric DTHP forms are expressed by two-dimensional ($\Phi$, $\Phi'$) maps, calculated in 10° steps. When calculating the ($\Phi$, $\Phi'$) maps, constant geometry based on the geometry of 2-methoxytetrahydropyran (MTHP) was used.

The regions of local minima on ($\Phi$, $\Phi'$) maps were used as starting points for optimization of the geometry. Molecular geometry and energies of the minima were obtained by optimizing 39 geometrical parameters, using the PCILO quantum-

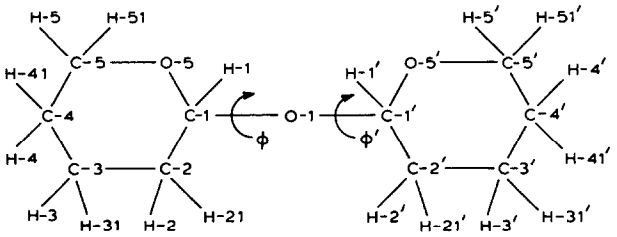


Fig 1 Numbering scheme of atoms in 2-(tetrahydropyran-2-yloxy)tetrahydropyran

chemical method[9]. Of the 87 geometrical parameters (bond lengths, bond angles, and torsion angles of all atoms), 39 were optimized, leaving out all parameters of the 16 hydrogen atoms, namely, those linked to C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, and C-5′. The molecular structure of the most populated conformers for each isomer was also optimized by MM2CARB, which is an MM2 force-field program[10] modified with the Jeffrey–Taylor acetal-segment parameters[11]. To obtain better agreement between calculated C–O bond lengths of oligosaccharides and those observed by X-ray or neutron-diffraction methods the following Jeffrey–Taylor equilibrium bond-lengths for the stretching-energy term were changed: C-1–O-5, from 140.0 to 142.2 pm; C-1–O-1, from 136.3 to 138.8 pm; and C-5–O-5, from 140.2 to 142.0 pm, for an equatorial anomeric group, and C-1–O-5 from 139.6 to 140.6 pm, C-1–O-1 from 138.0 to 139.6 pm, and C-5–O-5 from 141.2 to 142.6 pm for an axial anomeric group.

The method of free-energy calculation of individual conformers in dilute solution has been described in detail in our previous papers[12,13] and therefore, in the present work, we shall repeat only the basic principles of the model. The process of dissolving a molecule in a solvent involves two steps. The first is the creation, in the solvent, of a cavity of sufficient size to accommodate the solute molecule in the given conformation. The cavity formation requires a Gibbs free energy $G_{cav}$. The second step is the introduction into this cavity of the solute molecule, which then interacts with the surrounding solvent molecules. In our model, the interaction part of the solvation free energy, $G_{solv}$, is composed of the free energy of dispersion, $G_{disp}$, and electrostatic, $G_{elst}$, interactions. The total free energy $G_T$ of the individual conformers with corresponding intramolecular energy $G_u$ in the given solvent can be written as

$$G_T = G_u + G_{solv} = G_u + G_{cav} + G_{elst} + G_{disp}.$$

The calculation of the cavity term is based on an expression taken from the Scaled Particle Theory, which has been successfully used in studies of the thermodynamic properties of aqueous and nonaqueous solutions[14]. The electrostatic term is calculated according to Onsager's theory of the reaction field as applied by Abraham and Bretschneider[15]. The dispersion interactions take into account both attractive and repulsive nonbonded interaction, using a combination of the London dispersion equation and Born-type repulsion[12]. Expressions for the individual terms of the solvation free-energy function have been given, together with all necessary solvent parameters in our previous work[12,13]. Energy ($G_u$) and parameters characterizing the dimer conformations were calculated by the PCILO quantum-chemical method, with the expection of the refractive index and the hard-sphere radius. For the refractive index, we used the value[16], for the $\alpha,\alpha$-form, of 1.4600. The hard-sphere radius of each DTHP conformer was calculated from the van der Waals volume by using a program written by Pavani and Ranghino[17]. The van der Waals radii values of 140, 160, and 120 nm were respectively used for oxygen, carbon, and hydrogen atoms.

The orientation around the glycosidic bond may be experimentally evaluated from long-range, carbon–proton coupling-constants[18] and linkage rotation[19]. The value of the linkage rotation is directly related to the torsion angles $\Phi$ and $\Phi'$ by the equation $\Lambda = -105 - 120(\sin \Phi + \sin \Phi')$ and $\Lambda = 105 - 120(\sin \Phi + \sin \Phi')$ for the $\alpha$ and $\beta$ linkages, respectively[19]. Recently, Hamer and co-workers[18] published the angular dependence of the $^3J_{CH}$ coupling constant in the form of a plot of $J^{\Phi}$ *versus* torsion angle $\Phi$. This curve can be represented by the equation $J^{\Phi} = -0.13(1 - \cos \Phi) - 2.80(1 - \cos 2\Phi) + 0.53(1 - \cos 3\Phi) + 5.32$. From these equations, values for individual conformers were estimated, and then the average values $\langle P \rangle$ of a parameter $P$ ($J^{\Phi}$, $J^{\Psi}$, $\Lambda$) were calculated from the following equation

$$\langle P \rangle = \sum_{\Phi} \sum_{\Phi'} P(\Phi, \Phi') \exp [-\Delta G(\Phi, \Phi')/RT]/Q,$$

where $Q$ is the partition function of DTHP

$$Q = \sum_{\Phi} \sum_{\Phi'} \exp [-\Delta G(\Phi, \Phi')/RT].$$

## RESULTS AND DISCUSSION

*The α,α-form of 2-(tetrahydropyran-2-yloxy)tetrahydropyran* (AA-DTHP). — Fig. 2 shows conformational energy ($\Phi$, $\Phi'$) maps for an isolated molecule (Fig. 2a) and its aqueous solution (Fig. 2b). The contour maps demonstrate how both the exo-anomeric effect and steric interactions between the pyranoid-ring atoms

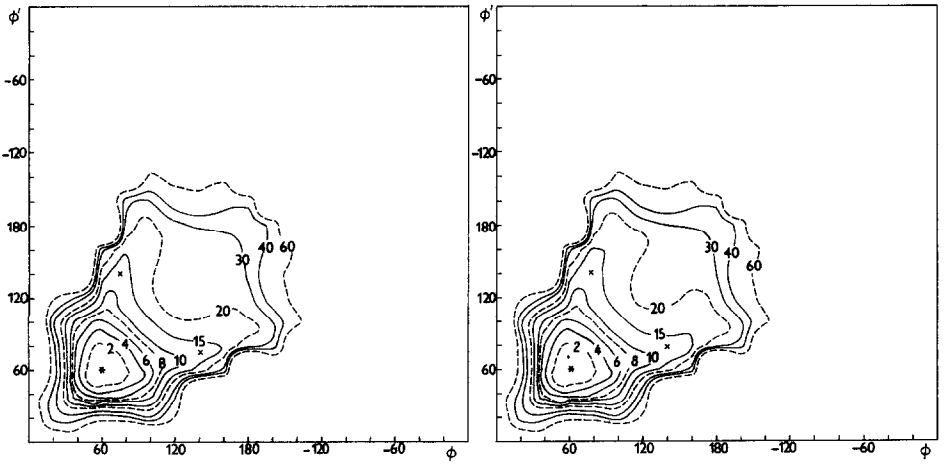


Fig 2 Energy surface (kJ/mol) for AA-DTHP as a function of torsion angles $\Phi$ and $\Phi'$ [(a) Isolated molecule, and (b) aqueous solution Key * indicates the lowest calculated minimum, x indicates calculated local minima ]

influence the rotation around the glycosidic C–O bonds. Only a small percentage of the conformational space lies within the 20 kJ/mol region. An inspection of the ($\Phi$, $\Phi'$) maps reveals the existence of three minima. Minima corresponding to the three conformers (sc,sc), (ap,sc), and (sc,ap) were used as starting points for PCILO and MM2CARB minimization. PCILO calculated energies and selected geometrical parameters characterizing optimal conformers, together with dipole moments and estimated values of the linkage rotation $\Lambda$, proton–carbon coupling constant $J^{\Phi}$, $J^{\Phi'}$, are listed in Table I.

For comparison, geometrical parameters of the MM2CARB lowest minimum and $\alpha,\alpha$-trehalose crystal structures are also included. The lowest energy is found for conformer (sc,sc), having $\Phi$ = 63.4 and $\Phi'$ = 59.1° (designated AA1). The (ap,sc) conformer (AA2) with $\Phi$ = 145.1, $\Phi'$ = 67.5° lies 9.87 kJ/mol higher in energy than the AA1 conformer. The symmetrically related conformation (sc,ap) with $\Phi$ = 67.5°, $\Phi'$ = 145.1 (AA3) has the same energy. The AA1 conformer corresponds to the crystal-structure conformation of $\alpha,\alpha$-trehalose[3–5]. In the MM2CARB structure, the molecule has $C_2$ symmetry and there is no significant difference in molecular dimensions of the anhydrous $\alpha,\alpha$-trehalose in the crystal[5] and that calculated by the modified MM2CARB method. The two glycosidic-

TABLE I

PCILO CALCULATED ENERGIES $\Delta E$ (kJ/mol), DIPOLE MOMENTS $\mu$(D), CARBON-PROTON COUPLING CONSTANTS $J^{\Phi}$, $J^{\Phi'}$ Hz, LINKAGE ROTATION $\Lambda$ (DEG), AND SELECTED GEOMETRICAL PARAMETERS (LENGTHS IN pm, ANGLES IN DEG) FOR OPTIMAL AA-DTHP CONFORMERS, COMPARED WITH MM2CARB VALUES AND EXPERIMENTAL VALUES OF CONFORMATIONS OF CRYSTAL $\alpha,\alpha$-TREHALOSE

| *Parameter* | *PCILO* | | | *α,α-Trehalose* | |
|---|---|---|---|---|---|
| | *AA1* | *AA2* | *MM2CARB* | *Exp.*[5] | *Exp* [3,4] |
| $\Delta E$ | 0.0 | 9 87 | | | |
| $\Phi$ | 63.6 | 145 1 | 61 4 | 60 8 | 74.8 |
| $\Phi'$ | 59.1 | 67 5 | 61 4 | 60 1 | 61.7 |
| $\Phi^{H}$ | −50 5 | 29.6 | −54.8 | | |
| $\Phi^{H'}$ | −54 6 | −47 1 | −54 8 | | |
| C-5–O-5 | 139.5 | 139 5 | 143 7 | 145 1 | 143 3 |
| O-5–C-1 | 139.8 | 139 7 | 141 4 | 141 9 | 142.1 |
| C-1–O-1 | 139.6 | 139 8 | 140 4 | 141 6 | 141.5 |
| O-1–C-1′ | 139.5 | 139 4 | 140.4 | 142 0 | 142.2 |
| C-1′–O-5′ | 139.8 | 139 8 | 141.4 | 142 1 | 140.4 |
| O-5′–C-5′ | 139 6 | 139 6 | 143 7 | 144 2 | 143 1 |
| C-5–O-5–C-1 | 111.6 | 111.5 | 111 8 | 114.3 | 114 0 |
| O-5–C-1–O-1 | 110 4 | 106.1 | 112 9 | 112.0 | 112 1 |
| C-1–O-1–C-1′ | 111 0 | 112 3 | 114 8 | 113 3 | 115 8 |
| O-1–C-1′–O-5′ | 110 8 | 110 9 | 112 9 | 111 9 | 111 7 |
| C-1′–O-5′–C-5′ | 111 5 | 111 9 | 111 8 | 115 5 | 114 2 |
| $\mu$ | 1 99 | 1.97 | | | |
| $J^{\Phi}$ | 2 91 | 4 43 | | | |
| $J^{\Phi'}$ | 2 56 | 3 20 | | | |
| $\Lambda$ | 85 4 | 42 2 | | | |

linkage torsion angles differ more in the PCILO calculated structure. Because the PCILO method applies a CNDO Hamiltonian, a small systematic difference in the bond lengths is to be expected[20], and this was observed; the C–C bonds are ~3 and the ring C–O bonds ~4 pm shorter than the experimental values. However, a larger difference does not exceed 3%. The bond angle C-5–C-1–O-1 is 110.4° in the AA1 conformer ($\Phi$ = 63.6°) *versus* 106.1° in the AA2 conformer ($\Phi$ = 145.1°). This difference is a clear manifestation of the exo-anomeric effect on the geometry of the acetal segment.

A comparison of the map for AA-DTHP in solution with that for an isolated AA-DTHP molecule shows only a minor alteration in the character of the maps as a result of the solvent effect. This is demonstrated in Fig. 2b for aqueous solution. The minima in solution and energy differences are the same as those for an isolated molecule. Such solution behavior is significantly different from the solution behavior of other saccharides, where marked differences in the solvent effect of aqueous and nonaqueous solutions on the orientational equilibrium around glycosidic linkage have been observed[12,13,21]. The calculated population of the AA1 conformer is 96.5%, both in an isolated molecule and in solution, as well as the average coupling constants $\langle J^{\Phi}\rangle$ = 2.9 Hz, $\langle J^{\Phi'}\rangle$ = 2.6 Hz, and the linkage rotation $\langle\Lambda\rangle$ = 83.9°. The average values calculated from the whole maps are similar $\langle J^{\Phi}\rangle$ = $\langle J^{\Phi'}\rangle$ = 2.6 Hz, $\langle\Lambda\rangle$ = 82°. The restricted motion around the glycosidic linkage in the AA-DTHP molecule is clearly demonstrated by the value of the partition function $Q$ = 3.22. The calculated average proton–carbon constant can be compared with the values $J^{\Phi}$ = $J^{\Phi'}$ = 3.0 Hz observed for $\alpha$-D-glucopyranosyl $\alpha$-D-mannopyranoside in water solution[8].

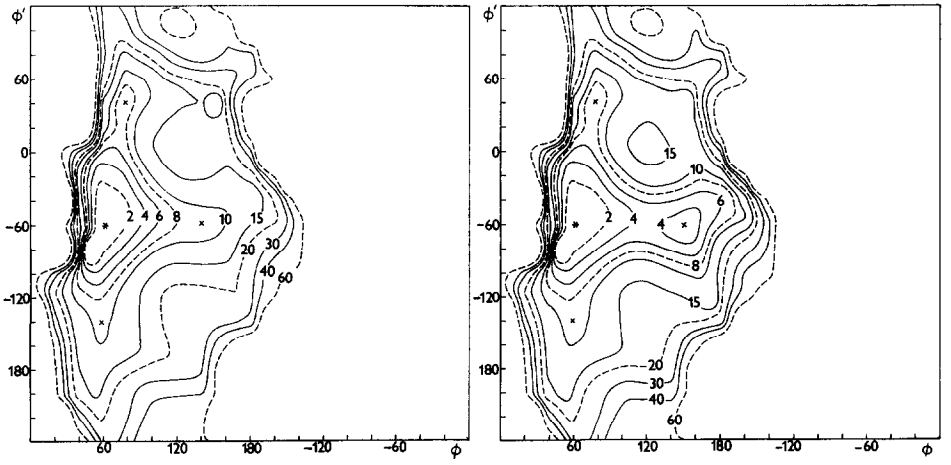


Fig 3 Energy surface (kJ/mol) for AB-DTHP as a function of torsion angles $\Phi$ and $\Phi'$ [(a) Isolated molecule, and (b) aqueous solution Key * indicates the lowest calculated minimum, x indicates calculated local minima ]

*The α,β-form of 2-(tetrahydropyran-2-yloxy)tetrahydropyran* (AB-DTHP). — Two-dimensional ($\Phi$, $\Phi'$) contour maps for an isolated AB-DTHP molecule and a water solution thereof are given in Fig. 3. It is evident form comparison of the AB-DTHP maps with the AA-DTHP maps that the change in the anomeric configuration at one anomeric carbon atom broadens the area enclosed by the contour 20 kJ/mol. The rotation around the equatorially oriented C-1′–O-1 bond is less hindered than that about the axially oriented C-1–O-1 bond. The maps show the existence of four minima (sc, −sc), (sc,ap), (sc,sc), and (ap, −sc), which were minimized to four conformers whose important characteristics are summarized in Table II. The lowest minimum AB1 appeared at $\Phi$ = 65.4° and $\Phi'$ = −54.7°, the next AB4, with ~5.1 kJ/mol higher energy at (151.5, −58.4). The energies of the AB2 (61.9, −147.8) and AB3 (72.5, 46.3) conformers relative to AB1 are 5.6 and 5.3 kJ/mol, respectively.

The conformational maps of AB-DTHP in solution preserve the overall shape of the map for an isolated molecule. However, with increased polarity of the solvent, the accessibility of the conformational space increases as well, especially in the areas corresponding to ap conformations. An enhanced flexibility of AB-DTHP in comparison with AA-DTHP is also reflected in calculated values of the partition

TABLE II

PCILO CALCULATED ENERGIES $\Delta E$ (kJ/mol), DIPOLE MOMENTS $\mu$(D), CARBON-PROTON COUPLING CONSTANTS $J^{\Phi}$, $J^{\Psi}$ LINKAGE ROTATION $\Lambda$ (DEG), AND SELECTED GEOMETRICAL PARAMETERS (LENGTHS IN pm, ANGLES IN DEG) FOR OPTIMAL AB-DTHP CONFORMERS, COMPARED WITH MM2CARB VALUES

| *Parameter* | *PCILO* | | | | *MM2CARB* |
|---|---|---|---|---|---|
| | *AB1* | *AB2* | *AB3* | *AB4* | |
| $\Delta E$ | 0.0 | 5 62 | 5.25 | 5 10 | |
| $\Phi$ | 65 4 | 61 9 | 72 5 | 151.5 | 60.1 |
| $\Phi'$ | −54.7 | −147 8 | 46 3 | −58 4 | −60 9 |
| $\Phi^{H}$ | −49 1 | −52 2 | −41 9 | 39 2 | −53.2 |
| $\Phi^{H'}$ | 62 2 | −32.2 | 160.9 | 58 3 | 55 1 |
| C-5–O-5 | 139 5 | 139 6 | 139 6 | 139 5 | 143 8 |
| O-5–C-1 | 139 7 | 139.8 | 139.8 | 139 7 | 141 6 |
| C-1–O-1 | 139 7 | 139 4 | 139 5 | 139 8 | 141 3 |
| O-1–C-1′ | 139.3 | 139 6 | 139 5 | 139 2 | 140 6 |
| C-1′–O-5′ | 139 8 | 139 8 | 139 9 | 139 9 | 143 6 |
| O-5′–C-5′ | 139 5 | 139.5 | 139.5 | 139 5 | 143 7 |
| C-5–O-5–C-1 | 111 4 | 113 3 | 111 8 | 110.9 | 112 4 |
| O-5–C-1–O-1 | 110 9 | 104 6 | 110 6 | 106 4 | 114 6 |
| C-1–O-1–C-1′ | 110 8 | 110 8 | 112 4 | 110 8 | 116 0 |
| O-1–C-1′–O-5′ | 107.0 | 102 8 | 107 1 | 106 9 | 110.5 |
| C-1′–O-5′–C-5′ | 111 3 | 111 8 | 110 9 | 111 4 | 112 8 |
| $\mu$ | 1 50 | 1 41 | 1 41 | 3.41 | |
| $J^{\Phi}$ | 3 04 | 2 77 | 3.61 | 3.81 | |
| $J^{\Psi}$ | 1 91 | 4.28 | 5 26 | 2.24 | |
| $\Lambda$ | −15 4 | 158 8 | 40 9 | −177.9 | |

function $Q$ (see Table III). The partition function is 3.2 in AA-DTHP *versus* 5.4 in isolated AB-DTHP and 7.4 in water solution of AB-DTHP. Although the minima are the same as those for an isolated molecule, the relative energies of individual conformers show significant differences. Calculated values of the molar fractions of the four AB-DTHP conformers, average carbon–proton constants and linkage rotation are listed in Table III. The distribution of conformers for the isolated molecule is AB1:AB2:AB3:AB4 = 74:8:9:9. In solution, the abundance of the AB1 conformer decreases as the polarity of the solvent increases, with the lowest value of 33% in water. In contrast, the abundance of the AB4 conformer increases to 58% in water. The populations of AB2 and AB3 change only little. The solvent effect is most pronounced in water. Whereas, in other solvents, AB1 is the principal conformer, in aqueous solution AB4 is predicted to be the principal species. The changes in the abundance of conformers are also reflected in the average values. The average value of the coupling constant $\langle J^{\Phi} \rangle$ increases from 3.2 Hz for an isolated molecule to 3.5 Hz in water, as well as the average linkage rotation, from $-120$ to $-199°$. In contrast, the average value of $\langle J^{\Phi'} \rangle$ is constant. The average values calculated from the whole maps show a similar trend, although the absolute values are smaller. For example, the average coupling constant $\langle J^{\Phi} \rangle$ is 2.9 Hz for an isolated molecule and 3.2 Hz for aqueous solution.

*The β,β-form of 2-(tetrahydropyran-2-yloxy)tetrahydropyran* (BB-DTHP). — For the BB-DTHP conformational analysis, we followed the same procedure used with the other two isomers. The calculated two-dimensional maps, given in Fig. 4, contain six local minima (−sc, −sc), (−sc,sc), (−sc,ap), (sc, −sc), (sc,sc), and (ap, −sc). The characteristics of the optimal conformers are listed in Table IV. The lowest minimum BB1 appeared at $\Phi = -58.6°$ and $\Phi' = -59.0°$, and it has $C_2$

TABLE III

CALCULATED MOLAR FRACTIONS, $x_i$, OF OPTIMAL CONFORMERS OF AB-DTHP, AND CALCULATED AVERAGE VALUES OF CARBON–PROTON CONSTANTS $\langle J^{\Phi} \rangle$, $\langle J^{\Phi'} \rangle$, LINKAGE ROTATION $\langle \Lambda \rangle$, AND PARTITION FUNCTION ($Q$) IN VARIOUS SOLVENTS

| | $x_i$ | | | | $\langle J^{\Phi} \rangle$ | $\langle J^{\Phi'} \rangle$ | $\langle \Lambda \rangle$ | Q |
|---|---|---|---|---|---|---|---|---|
| | *AB1* | *AB2* | *AB3* | *AB4* | | | | |
| Isolated molecule | 74 0 | 7 7 | 8 9 | 9 4 | | | | |
| 1,4-Dioxane | 68 1 | 8.9 | 10 1 | 12 9 | 3 2 | 2.5 | −15 3 | 5 42 |
| Carbon tetrachloride | 69 2 | 8 7 | 9 9 | 12 2 | 3 2 | 2 5 | −14 5 | 5 31 |
| Chloroform | 64 8 | 8 3 | 9 4 | 17 5 | 3 2 | 2 5 | −24 0 | 5 60 |
| Pyridine | 63 3 | 7 8 | 8.9 | 20 0 | 3 2 | 2 5 | −29 2 | 5 58 |
| Acetone | 63 8 | 7 6 | 8 9 | 19 7 | 3 2 | 2 5 | −29 1 | 5 58 |
| Methanol | 57 4 | 6 8 | 7 9 | 27 9 | 3 3 | 2 4 | −44 6 | 5 85 |
| Acetonitrile | 59 6 | 7 3 | 8 3 | 24 8 | 3 3 | 2 4 | −38 4 | 5 76 |
| Dimethyl sulfoxide | 62 2 | 7 4 | 8 5 | 21 9 | 3 2 | 2 4 | −33 2 | 5 62 |
| Water | 33 4 | 4 1 | 4 7 | 57 8 | 3 5 | 2 4 | −93 5 | 7 41 |
| *N*-Methylacetamide | 51 5 | 5 9 | 6 8 | 35 8 | 3 3 | 2 4 | −59 4 | 6 16 |

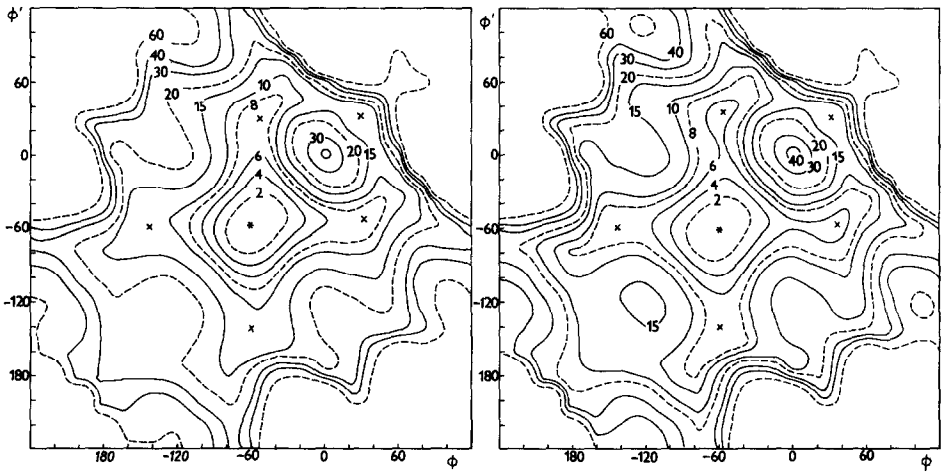


Fig 4 Energy surface (kJ/mol) for BB-DTHP as a function of torsion angles $\Phi$ and $\Phi'$ [(a) Isolated molecule, and (b) aqueous solution Key * indicates the lowest calculated minimum; x indicates calculated local minima ]

TABLE IV

PCILO CALCULATED ENERGIES $\Delta E$ (kJ/mol), DIPOLE MOMENTS $\mu$(D), CARBON-PROTON COUPLING CONSTANTS $J^{\Phi}$, $J^{\Phi'}$ LINKAGE ROTATION $\Lambda$ (DEG), AND SELECTED GEOMETRICAL PARAMETERS (LENGTHS IN pm, ANGLES IN DEG) FOR OPTIMAL BB-DTHP CONFORMERS, COMPARED WITH MM2CARB VALUES

| *Parameter* | *PCILO* | | | | *MM2CARB* |
|---|---|---|---|---|---|
| | *BB1* | *BB2* | *BB4* | *BB6* | |
| $\Delta E$ | 0.0 | 4 28 | 7 83 | 6 75 | |
| $\Phi$ | −58.6 | 59 0 | 34 6 | −60 0 | |
| $\Phi'$ | −59.0 | −39.3 | −55 9 | 34 5 | −59 9 |
| $\Phi^{H}$ | 58.2 | 173 4 | −33 4 | 149 6 | 56 9 |
| $\Phi^{H'}$ | 57 8 | 77 5 | 60 8 | 149 3 | 57.1 |
| C-5–O-5 | 139 5 | 139.5 | 139 5 | 139 5 | 143.1 |
| O-5–C-1 | 139 9 | 139 8 | 139 8 | 139 8 | 142 9 |
| C-1–O-1 | 139 3 | 139 5 | 139 6 | 139 5 | 139 7 |
| O-1–C-1′ | 139 3 | 139 3 | 139 2 | 139 5 | 139 7 |
| C-1′–O-5′ | 139 9 | 139 8 | 139 9 | 139 8 | 142 9 |
| O-5′–C-5′ | 139 5 | 139.5 | 139 6 | 139 6 | 143 1 |
| C-5–O-5–C-1 | 111 5 | 110 7 | 111 6 | 111 1 | 110 9 |
| O-5–C-1–O-1 | 106 4 | 106 8 | 102 2 | 107 9 | 108 4 |
| C-1–O-1–C-1′ | 110 1 | 111 4 | 111 2 | 111 1 | 112 9 |
| O-1–C-1′–O-5′ | 106 4 | 107 5 | 106 9 | 107 8 | 108 5 |
| C-1′–O-5′–C-5′ | 111 4 | 111 2 | 112 0 | 111 1 | 110 9 |
| $\mu$ | 1 38 | 2 17 | 3 01 | 1 48 | |
| $J^{\Phi}$ | 2 25 | 5 99 | 3 98 | 4 13 | |
| $J^{\Phi'}$ | 2 29 | 0 71 | 2 03 | 4 10 | |
| $\Lambda$ | −98 5 | −25 9 | 66 3 | −16 4 | |

symmetry, as well as the BB6 conformer (34.6, 34.6) with ~6.8 kJ/mol higher energy. The energy of the BB2 (59.0, −39.3) conformer and of the symmetrically related BB3 (−39 3, 59.0) conformer, relative to BB1, is 4.3 kJ/mol. The last two symmetrical conformers, BB4 (−33.4, 60.8) and BB5 (60.8, −33.4), are situated at 7.8 kJ/mol above the absolute minimum. The distribution of conformers for the isolated molecule is BB1:(BB2 + BB3):(BB4 + BB5):BB6 = 66:24·6:4

The conformational surface of BB-DTHP, having both anomeric C–O bonds oriented equatorially, is more complex than those for AA-DTHP or AB-DTHP. The area lying within the 20 kJ/mol region in BB-DTHP exceeds that in AA-DTHP by a factor of 2. An increased flexibility around the glycosidic linkage in BB-DTHP is also demonstrated by the value of the partition function, 6.3 for an isolated BB-DTHP, which is double the value for AA-DTHP. The conformational maps of BB-DTHP in solution preserve the symmetry of the maps observed for an isolated molecule. The solvent effect manifests itself mainly in the change of relative energies of the individual conformers. However, with increased polarity of the solvent, the flexibility of the glycosidic linkage is also enhanced; this is reflected in the values of the partition function (see Table V). The calculated molar fractions listed in Table V show that the equilibrium composition is solvent-dependent. The abundance of the BB1 conformer decreases with an increase in solvent polarity, with the lowest value of 47% in water, but it remains as the global minimum. In contrast, the abundance of BB2 (BB3) and BB4 (BB5) increases to 14 and 11%, respectively, and that of the BB6 conformer decreases slightly. The change in equilibrium composition of BB-DTHP is reflected in the average coupling constant to only a slight degree. Carbon–proton coupling constants are $\langle J^{\Phi}\rangle = \langle J^{\Psi}\rangle = 2.6$ Hz in the isolated molecule, and $\langle J^{\Phi}\rangle = \langle J^{\Psi}\rangle = 2.8$ Hz in water. On the other hand,

TABLE V

CALCULATED MOLAR FRACTIONS, $x_i$, OF OPTIMAL CONFORMERS OF BB-DTHP, AND CALCULATED AVERAGE VALUES OF CARBON-PROTON CONSTANTS $\langle J^{\Phi}\rangle$, $\langle J^{\Psi}\rangle$, LINKAGE ROTATION $\langle \Lambda \rangle$, AND PARTITION FUNCTION ($Q$) IN VARIOUS SOLVENTS

| | $x_i$ | | | | $\langle J^{\Phi}\rangle$ | $\langle J^{\Psi}\rangle$ | $\langle \Lambda \rangle$ | Q |
|---|---|---|---|---|---|---|---|---|
| | *BB1* | *BB1, BB3* | *BB4, BB5* | *BB6* | | | | |
| Isolated molecule | 66 3 | 11 8 | 2 9 | 4 3 | | | | |
| 1,4-Dioxane | 68 6 | 10 3 | 3 4 | 4 1 | 2 6 | 2 6 | −69 0 | 6 32 |
| Carbon tetrachloride | 68 4 | 10 5 | 3 2 | 4 2 | 2 6 | 2 6 | −69 2 | 6 44 |
| Chloroform | 65 9 | 10 9 | 4 1 | 3 9 | 2 6 | 2 7 | −65.8 | 6 66 |
| Pyridine | 64.0 | 11 5 | 4 5 | 3 9 | 2 7 | 2 7 | −63 6 | 6 76 |
| Acetone | 63 8 | 11 6 | 4 4 | 4 0 | 2 7 | 2 7 | −63 6 | 6 79 |
| Methanol | 59 1 | 12 8 | 5 8 | 3 8 | 2 7 | 2 7 | −57 8 | 7 22 |
| Acetonitrile | 61 2 | 12 2 | 5 3 | 3 9 | 2 7 | 2 7 | −60 2 | 7 02 |
| Dimethyl sulfoxide | 62 0 | 12 2 | 4 8 | 3 9 | 2 7 | 2 7 | −61 7 | 6 97 |
| Water | 47 4 | 14 1 | 10 7 | 3 1 | 2 8 | 2 8 | −40 3 | 8 81 |
| *N*-Methylacetamide | 55 2 | 13 6 | 7 0 | 3 6 | 2 7 | 2 7 | −52 6 | 7 65 |

the average linkage rotation changes from −69° for an isolated molecule to −40° in water.

It follows from our results that the rotation around the C–O bond in (1↔1)-linked disaccharides depends on the orientation of the adjacent C–O bonds in the C–O–C–O–C–O–C segment. The orientation of the C-1–O-5 or C-1′–O-5′ bond is determined by the configuration of the anomer; for the $\alpha$ anomer (or an axial form in DTHP) the orientation is sc; for the $\beta$ anomer (or an equatorial form in DTHP) the orientation is ap. The shape of the energy curve for the rotation around the C-1–O-1 or C-1′–O-1′ bond resembles that in the axial (A-MTHP) and equatorial (E-MTHP) forms of 2-methoxytetrahydropyran[22]. For A-MTHP, the existence of two minima for rotation around the C–O bond has been found. For E-MTHP, three minima occurred.

The same situation obtains in the case of DTHP. The calculations revealed the existence of two minima for rotation around the axially oriented C-1–O-1 bond, and three minima for the equatorially oriented C-1–O-1 bond. On the other hand, the orientation around the C-1–O-1 or C-1′–O-1 bond influences the rotation about the adjacent C-1′–O-1 or C-1–O-1 bond. Therefore, we have found only three minima for AA-DTHP, four for AB-DTHP, and six for BB-DTHP. Disaccharides usually exist in a complex, conformational equilibrium in solution, and the composition of the mixture of conformers depends on the solvent. In this respect, the solution properties of the $\alpha,\alpha$-form of (1↔1)-linked disaccharides are unique. As follows from our results, ~96% of the AA-DTHP is in the AA1 conformation, and the abundance of AA1 is not solvent-dependent.

*Anomeric and exo-anomeric effects in DTHP.* — The electronic structure of the acetal segment C-5–O-5–C-1–O-1–C in glycosides affects the geometry and conformation of a molecule, the overall consequences being termed anomeric and exo-anomeric effects[23]. These effects are related to the favoring of a synclinal arrangement around two C–O bonds, O-5–C-1 and C-1–O-1, respectively. In (1↔1)-linked disaccharides, the C-5–O-5–C-1–O-1–C-1′–O-5′–C-5′ segment is present, and thus, the properties of four C–O bonds are affected. The anomeric effect is related to the orientational preference around O-5–C-1 and O-5′–C-1′ bonds, whereas the exo-anomeric effect influences the orientational properties of C-1–O-1 and O-1–C-1′ bonds.

The results of this study make it possible to calculate the solvent effect on the equilibrium composition of the 13 DTHP conformers, and to estimate the magnitude of the anomeric and exo-anomeric effects. For the purpose of this investigation, we shall define the anomeric and exo-anomeric effects as the difference between the free energies of the conformers having the corresponding bond in the sc or ap position. In evaluating the anomeric and exo-anomeric effects in DTHP, several different equilibria must be considered separately. Table VI shows how the magnitude of the theoretical anomeric and exo-anomeric effects in the DTHP depends on the solvent. The magnitude of the anomeric effect is in the range of 0.5–4 9 kJ/mol, and that of the exo-anomeric effect varies between 1.5 and 10.1

TABLE VI

THE EFFECT OF SOLVENTS UPON THE ANOMERIC AND EXO-ANOMERIC EFFECTS (kJ/mol)

| *Solvent* | *Anomeric effect* | | | *Exo-anomeric effect* | | | |
|---|---|---|---|---|---|---|---|
| | *AB-BB* | *AA-AB* | *AB-BB* | *AA* | *BB* | $AB_A$ | $AB_\beta$ |
| Isolated molecule | 4 3 | 3 4 | 0 9 | 9 9 | 8 7 | 5 6 | 6.2 |
| 1,4-Dioxane | 4 6 | 4 1 | 0 5 | 10 1 | 8 3 | 5 8 | 4 9 |
| Carbon tetrachloride | 4.6 | 4 0 | 0 6 | 10 1 | 8 4 | 5 8 | 4 9 |
| Chloroform | 4 7 | 4 1 | 0 6 | 10 1 | 7 8 | 5 9 | 3 8 |
| Pyridine | 4 7 | 4 0 | 0 7 | 10 1 | 7 6 | 6 1 | 3 5 |
| Acetone | 4 7 | 4 0 | 0 7 | 10 1 | 7 5 | 6 1 | 3 5 |
| Methanol | 4 7 | 3 9 | 0 8 | 10 1 | 6 9 | 6 5 | 2 3 |
| Acetonitrile | 4 7 | 4 0 | 0 7 | 10 1 | 7 0 | 6 3 | 2 7 |
| Dimethyl sulfoxide | 4 7 | 3 9 | 0 8 | 10 1 | 7 4 | 6 3 | 3 2 |
| Water | 4 9 | 3 3 | 1 6 | 10 2 | 5 3 | 7 8 | −0 8 |
| *N*-Methylacetamide | 4 7 | 3 7 | 1 0 | 10 1 | 6 4 | 6 8 | 1 5 |

kJ/mol. However, in water, the AB-DTHP form does not exhibit any exo-anomeric effect for a rotation around the axially oriented C-1–O-1 bond. In 2-methoxytetrahydropyran, the magnitude of the anomeric and exo-anomeric effect in water, in comparison to that of those of the isolated molecule, decreases[12] by a factor of ½ to ¼, but, in the case of DTHP, we have not observed this trend. The exception is the exo-anomeric effect in the $AB_A$- and BB-form. In other cases, the magnitude of both effects increases in water. In the isolated molecule, the magnitude of the anomeric and exo-anomeric effects is determined by the equilibrium of intramolecular, electrostatic and delocalization interactions, with a predominant influence of the electrostatic interactions. Solute–solvent, electrostatic interactions are mainly responsible for an equilibrium shift in solution[12]. These interactions approximately follow the conformational dependence of the dipole moment. The magnitude of the dipole moment in DTHP is determined by the mutual orientation of the ring C–O and glycosidic C–O bonds. Because the value of dipole moments decreases in the order AB4 > BB4(BB5) ≃ BB2(BB3) ≃ AA1 ≃ AA2(AA3) > BB6 ≃ BB1 ≃ AB1 ≃ AB2 ≃ AB3, the stabilization decreases in the same order. It is evident from the values in Table VI that the anomeric and exo-anomeric effects are important driving-forces in the nonreducing disaccharides. In the case of the $\alpha,\beta$-form, these effects are even more pronounced in aqueous solution. The role of both effects is clearly documented in the global minimum AA1 of the 2-(tetrahydropyran-2-yloxy)tetrahydropyran, where all C–O bonds are in a synclinal position.

REFERENCES

1 J F KENNEDY AND C. A WHITE, *Bioactive Carbohydrates in Chemistry, Biochemistry and Biology*, Ellis Horwood, Chichester, 1983, pp 138–139
2 S W HUNTER, I JARDIN, D L YANAGIHARA, AND P J BRENNEN, *Biochemistry*, 24 (1985) 2798–2805

3 G. M BROWN, D C ROHRER, B BERKIN, C A BEEVERS, R. O. GOULD, AND R. SIMPSON, *Acta Crystallogr , Sect B,* 28 (1972) 3145–3158.
4 T TAGA, M. SENMA, AND K OSAKI, *Acta Crystallogr., Sect. B,* 28 (1972) 3258–3263.
5 G. A. JEFFREY AND R NANNI, *Carbohydr Res.,* 137 (1985) 21–30
6 S KOTO, S. INADA, AND S ZEN, *Chem Lett ,* (1980) 403–406
7 A A PAVIA, S N UNG-CHHUN, AND J M LACOMBE, *Nouv J Chim ,* 5 (1980) 101–108
8 K BOCK, J. DEFAYE, H DRIGUEZ, AND E. BAR-GUILLOUX, *Eur. J Biochem ,* 131 (1983) 595–600
9 S DINER, J. P. MALRIEU, F. JORDAN, AND M GILBERT, *Theor Chim. Acta,* 15 (1969) 100–110
10 N. L ALLINGER, *J Am Chem. Soc.,* 99 (1977) 8127–8134
11 G A. JEFFREY AND R. TAYLOR, *J. Comput Chem ,* 1 (1980) 99–109
12 I TVAROŠKA AND T KOŽÁR, *J Am. Chem Soc ,* 102 (1980) 6929–6936
13 I TVAROŠKA, *Biopolymers,* 23 (1982) 1887–1897.
14 R A PIEROTTI, *Chem Rev ,* 76 (1976) 717–726
15 R J ABRAHAM AND E BRETSCHNEIDER, in W J ORVILLE-THOMAS (Ed ), *Internal Rotation in Molecules,* Academic Press, London, 1974, Chapter 13
16 C. QUIVORON AND J NÉEL, *J Chem. Phys.,* 63 (1966) 1199–1209
17 R PAVANI AND G RANGHINO, *Comput Chem.,* 6 (1982) 133–135
18 G K HAMER, F BALZA, N CYR, AND A S PERLIN, *Can. J Chem ,* 56 (1978) 3109–3116
19 D A REES, *J Chem Soc , B,* (1970) 877–884
20 I TVAROŠKA AND T KOŽÁR, *J. Mol Struct ,* 123 (1985) 141–154.
21 I TVAROŠKA AND T KOŽÁR, *Theor Chim Acta,* 70 (1986) 91–114
22 I TVAROŠKA AND T. KOŽÁR, *Carbohydr Res ,* 90 (1981) 173–185.
23 R. U LEMIEUX AND S. KOTO, *Tetrahedron,* 30 (1974) 1933–1944