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CONFORMATIONS OF DISACCHARIDES BY EMPIRICAL, FORCE-FIELD CALCULATIONS: PART III, β-GENTIOBIOSE

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ABSTRACT

The conformational flexibility of β-gentiobiose has been studied by using convergent energy minimisation in a new force-field, with relaxation of all degrees of freedom. Twenty-four local minima are found in the ϕ,ψ,ω-space. The free-enthalpy differences are 1.7, 3.7, 5.1, 5.3, and from 5.9 to 29.4 kJ.mol^{-1} above the lowest minimum, corresponding to a distribution of 40:20:9:5:5:21 at 298 K. Each minimum is surrounded by a manifold of minimum conformers that differ only in exocyclic torsions. The average conformation of β-gentiobiose is not fully extended, but to some degree coiled. Three conformers are shown in stereo.

INTRODUCTION

In the previous papers[1,2] of this series, conformations and conformational interchange of β-maltose and β-cellobiose were described. In those studies, we used a simple force-field, where the parameters were taken from similar studies of α- and β-D-glucopyranose[3,4]. From theoretical considerations and the experience gained from the calculations on the disaccharides, we have now improved the force field. The three-parameter Buckingham potential is substituted by the two-parameter Lennard-Jones potential, and electrostatic interactions are included; reliable net-charges for mono- and di-saccharides are available from *ab initio* computations on D-glucopyranoses and β-maltose[5]. All parameters in the force-field are fitted to a series of test molecules consisting of normal and cyclic alkanes and ethers, alkanols, and saccharides. A detailed documentation of this new, consistent force-field is given elsewhere[6].

Force field. — The new force field FF400 is still a conventional one: harmonic functions for bond and angle deformations, Pitzer terms for torsional motions, and Lennard-Jones potentials and Coulomb terms for non-bonded interactions. All interactions separated by three or more bonds are considered to be non-bonded.

The force-field and parameters are given in Table I. As interactions between

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TABLE I

FORCE FIELD FF400[a]

Bond deformations:	$E_b = \sum_{\text{bonds}} \frac{1}{2}K_b(b - b_0)^2$	
	K_b	b_0
O-C	3012	1.410
C-C	2134	1.509
O-H	4477	0.955
C-H	2803	1.093
Angle deformations:	$E_\theta = \sum_{\text{angles}} \frac{1}{2}K_\theta(\theta - \theta_0)^2$	
	K_θ	$\theta_0{}^b$
O-C-O	251	
O-C-C	418	
O-C-H	418	
C-O-C	293	1.80
C-O-H	335	1.80
C-C-C	209	
C-C-H	297	
H-C-H	314	
Torsional terms:	$E_\theta = \sum \frac{1}{2}K_\phi(1 + \cos 3\phi)$	
	K_ϕ	
X-O-C-Y[c]	0.004	
X-C-C-Y	0.004	
Non-bonded interactions:		
Lennard-Jones terms:	$E_{LJ} = \sum_{i>j} \{\varepsilon_{ij}(r_{ij}^*/r_{ij})^{12} - 2\varepsilon_{ij}(r_{ij}^*/r_{ij})^6\}$	
	ε_{ij}	r_{ij}^*
O---O	1.255	3.00
O---C	1.255	3.05
C---C	0.418	3.50
O---H	0.418	2.95
C---H	0.418	3.15
H---H	1.255	2.75
Coulomb terms[d]:	$E_C = \sum_{i>j} F e_i e_j / D r_{ij}$	
	$e_i{}^e$	
O	−0.400	
C	−0.050	
H	0.125	

[a] Units are chosen so that energy is given in kJ.mol^{-1}; distances are in Å, angles in rad. [b] $\theta_0 = \arccos(-1/3)$ (= the tetrahedral angle), except where noted. [c] X and Y may be O, C, and H. [d] F = 1389.47; D = 3. [e] These are input values. The programme performs the following: (*1*) any atom is assigned the parameter value; (*2*) H on O is reassigned to −e(O); (*3*) the difference between the molecular charge (zero in this work) and the accumulated charge is distributed evenly on all atoms.

charge clouds on vicinal, sigma-bonded atoms are treated as pure, non-bonded interactions, the contribution from E_ϕ to the total energy E_T is very small due to very small values of K_ϕ. A special feature of our CFF-programme is the assignment of net charges. Charge parameters for chain and side-chain atoms, and hydrogen atoms bonded to carbon atoms, are needed; the programme automatically assigns net charges on hydrogen atoms bonded to oxygen atoms. Total charge neutralisation is made after the primary assignment. For disaccharides, this implies that the charge parameters shown in Table I will affect the following net-charge assignment:

$O = -0.400$, $C = -0.050$, $H(O) = 0.406$, and $H(C) = 0.125$.

The change in force-field has consequences for the calculated properties of α- and β-D-glucopyranose[3,4], β-maltose[1], and β-cellobiose[2] reported earlier. A detailed analysis of these consequences is reported elsewhere[7], but some main results are mentioned in the Appendix.

Nomenclature. — In the following, β-gentiobiose connotes 6-*O*-β-D-glucopyranosyl-β-D-glucopyranose. The constitution of β-gentiobiose, giving atom numbering and the torsional angles ϕ, ψ, and ω for later reference, is shown in Fig. 1; ϕ is defined by atoms H(C-1')–C-1'–O-6–C-6, ψ by C-1'–O-6–C-6–C-5, and ω by H(C-5)–C-5–C-6–O-6. Our definition of a torsional angle follows the IUPAC convention[8]: A torsional angle A–B–C–D is defined through a Newman projection; it is the angle through which A–B must be rotated around B–C to cover C–D when looking from B towards C; the sign is positive if the sense of rotation is clockwise.

Earlier calculations. — Using hard-sphere calculations, Rees and Scott[9] reached some general results concerning α- and β-(1→6) linkages of glucopyranose. They concluded that these polysaccharides are very flexible but, on average, rather extended, with ϕ and ω resembling the corresponding torsional angles in methyl glycosides, and $\psi \sim 180°$.

Mapping the three-dimensional ϕ,ψ,ω-space defined by α-(1→6)-linked, rigid

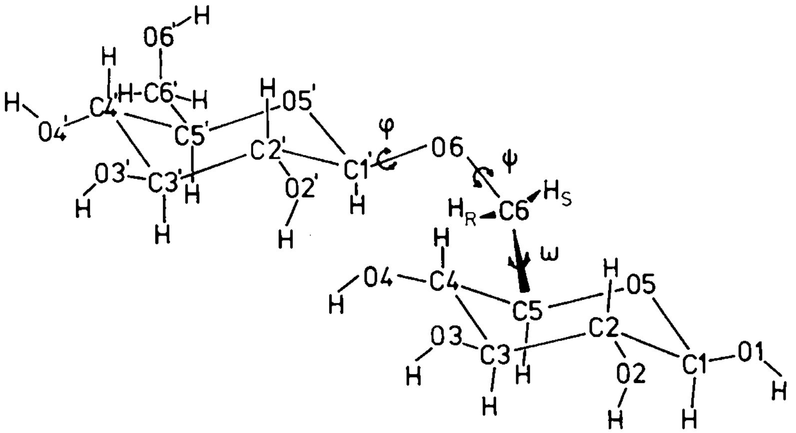


Fig. 1. Constitution and atom numbering for β-gentiobiose (6-*O*-β-D-glucopyranosyl-β-D-glucopyranose).

glucopyranose residues, Tvaroska *et al.*[10] found seven allowed minima differing by less than 10 kJ.mol^{-1} in potential energy. For the polymer, this corresponds to a broad range of helical dimensions, which explains the difficulty in obtaining oriented, crystalline fibers.

Experimental studies. — No crystal-structure analysis of β-gentiobiose has yet been made. Gagnaire *et al.*[11] measured carbon–carbon and carbon–proton coupling constants for mono- and di-saccharides selectively labeled at C-1, C-1′, and C-6. Furthermore, the two different methylenic protons at C-6 were assigned by stereospecific monodeuteration. Using a Karplus-type relationship between the observed coupling constants and conformation, they reached the following conclusions for β-gentiobiose octa-acetate dissolved in $CDCl_3$: $\phi \approx +10°$, $\psi \approx -130°$, and $\omega \approx -10°$, 180°, or $-60°$.

Bock and Vignon[12] performed proton-relaxation and Overhauser experiments on these compounds. The interpretation of the data gave interatomic distances between the protons involved in the rotations around ϕ, ψ, and ω. The nuclear Overhauser effect gave the qualitative conclusion that H_R(C-6) is closer to H(C-1′) than is H_S(C-6). From proton-relaxation measurements, they derived distances H_R(C-6)---H(C-1′) and H_S(C-6)---H(C-1′) in the range 2.5 to 2.7 Å, the former being the shorter. To explain these short distances, the values for ϕ and ψ found by Gagnaire *et al.*[11] seem to be reasonable. Relaxation and proton–proton coupling data[12] indicate that the two hydroxymethyl groups, on the average, have the same conformation, corresponding to ω-values in the range 120° to $-180°$.

CALCULATIONS

The computational methods and programmes used here were developed from the CFF system of Lifson and Warshel[13] by Niketić and Rasmussen[14]. Energy minimisation was performed by allowing all degrees of freedom to relax, *i.e.*, no internal co-ordinate was kept fixed. The minima on the potential-energy surface were then related to equilibrium conformations.

Initial conformations. — All initial conformations of β-gentiobiose were produced by suitable rotations around the bonds defining ϕ, ψ, and ω. Although the β-(1→6) linkage introduces one more rotational degree of freedom, increasing the theoretical number of initial conformations by one power, it has been possible, through the use of molecular models and chemical intuition, to limit the number of initial conformations to 27.

Energy minimisation. — The initial conformations were minimised through 20–40 steepest descent iterations followed by 10–15 steps of a modified Newton algorithm[14a]. Since the inclusion of Coulomb interactions causes the potential surface to be flatter, this minimisation strategy had to be repeated in some cases. Minimisation was considered finished when the quadratic norm of the gradient became less than 10^{-6} kJ.mol^{-1}.Å^{-1}.

TABLE II

GLYCOSIDIC TORSIONAL AND VALENCE ANGLES, DIFFERENCES IN FREE ENTHALPY ($T = 298$ K) AND TOTAL ENERGY, AND ENERGY CONTRIBUTIONS[a] FOR THE EQUILIBRIUM CONFORMERS OF β-GENTIOBIOSE

Minimum	ϕ	ψ	ω	*C-1′-O-6-C-6*	ΔG	ΔE_T	E_T	E_b	E_θ	E_{LJ}	E_C	E_{nb}
	(degrees)				*(kJ.mol^{-1})*							
1	23	91	−54	116.3	6.9	10.6	−97.2	7.4	38.6	−70.7	72.5	143.2
2	41	−96	47	115.3	5.3	10.5	97.3	7.5	38.3	66.9	76.2	143.1
3	42	64	68	116.4	11.1	13.6	−94.6	7.8	39.3	77.5	−64.2	−141.7
4	61	−118	177	115.0	9.4	15.0	−92.8	7.6	35.2	−66.7	−68.9	−135.6
5	64	120	55	114.7	0.0	0.0	−107.8	8.1	44.4	78.4	81.9	−160.3
6	75	−162	47	113.7	5.1	17.5	−90.3	7.3	33.6	60.5	−70.7	−131.2
7	79	95	133	115.3	5.9	1.3	−106.5	8.9	40.7	−85.2	−70.9	−156.1
8	94	−61	49	115.8	3.7	6.6	−101.2	8.0	41.0	73.4	76.8	−150.2
9	106	86	37	116.1	1.7	6.0	−101.8	7.6	39.0	77.5	70.9	−148.4
10	145	163	47	115.2	7.2	18.9	−88.9	7.4	36.0	−61.9	−70.4	−132.3
11	149	149	46	115.6	5.9	11.6	−96.2	7.6	38.2	68.5	73.4	−141.9
12	149	−93	16	116.3	7.2	9.2	−98.6	7.8	39.7	77.6	−68.5	−146.1
13	155	−80	80	117.3	8.2	10.8	−97.0	8.1	49.9	77.8	77.2	−155.0
14	164	122	45	116.7	9.3	17.3	90.5	7.6	39.2	65.5	71.8	−137.3
15	164	120	174	117.1	9.4	9.8	98.0	7.6	40.5	81.0	−65.1	−146.1
16	167	119	174	117.5	5.5	6.1	−101.7	7.7	40.9	−78.2	−72.1	−150.3
17	−3	72	158	117.0	12.1	10.5	−97.3	8.2	42.2	−79.1	68.6	−147.7
18	8	−157	−45	117.4	14.9	22.7	−85.1	7.5	39.7	−61.9	−70.4	−132.3
19	22	108	53	118.6	13.6	15.6	−92.2	7.9	45.2	74.8	70.5	−145.3
20	50	−112	55	118.1	22.2	22.8	−85.0	8.4	47.2	−78.3	62.3	−140.6
21	57	153	46	116.5	14.4	22.6	−85.2	7.5	44.4	−69.8	67.3	−137.1
22	−64	162	−47	116.3	22.6	31.8	−76.0	7.5	41.4	64.8	60.1	−124.9
23	65	160	155	116.7	24.9	29.8	−78.0	8.1	43.0	−66.3	−62.8	−129.1
24	−74	75	43	120.1	29.4	34.4	−73.4	8.0	52.8	71.0	63.2	−134.2

[a] In all conformers, the contribution from E_ϕ is less than 0.01 kJ.mol^{-1}.

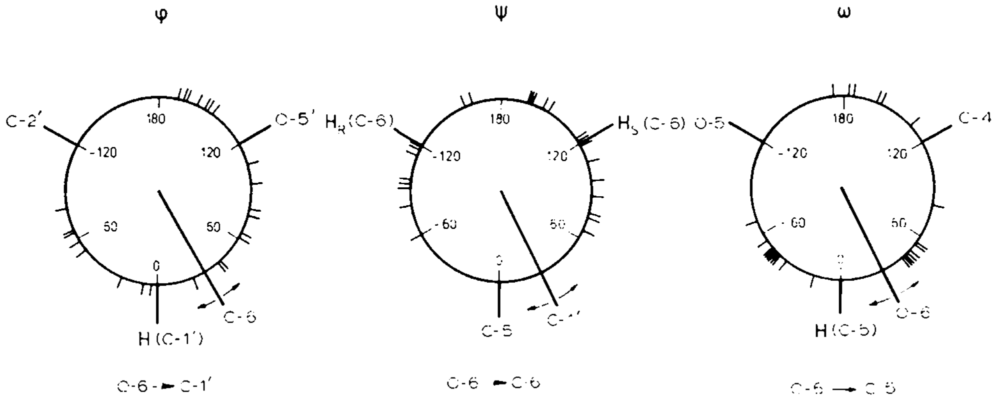


Fig. 2. Values of ϕ, ψ, and ω for the 24 local minima.

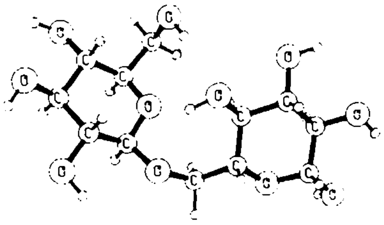
RESULTS AND DISCUSSION

The minima. — An expected, extreme, conformational flexibility is confirmed by the calculations. The 27 initials are minimised to 24 different local minima. For each minimum, different exocyclic torsions defining the orientation at the hydroxymethyl group and the hydroxyl groups that might be involved in intramolecular hydrogen-bonds have been examined, in order to find the optimal combination of these torsional angles. All minima are listed in Table II. In Fig. 2, all values of ϕ, ψ, and ω are plotted on Newman projections. The ϕ-values are distributed over a large range, but the cases of lowest energy are found in the interval 60° to 110°, except for a specimen near 170°; this is in accordance with the exo-anomeric effect[15]. The ψ-values are distributed over an interval ranging from +60° over 180° to −60°; the 120° interval from −60° over 0° to +60° is not occupied due to strong repulsion between two rings. The ω-values are not centered symmetrically around +60°, 180°, and −60°, mainly because of strong O---O repulsions (O-6---O-5 for 180° and −60°, and O-6---O-4 for +60°). Tvaroska *et al.*[10] excluded all minima of the α-(1→6)-linked disaccharide with ω-values between 0° and +120°. We cannot find sufficient experimental evidence for the β-(1→6) linkage to make a corresponding exclusion.

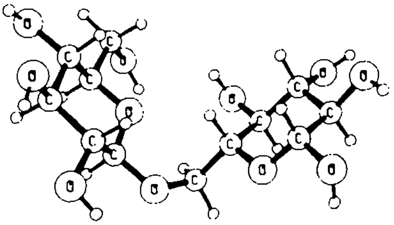
From Table II, it is seen that while E_b is rather constant, both E_θ and $E_{nb} = E_{LJ} + E_C$ are responsible for the energy differences between the minima. A coiled conformation is favoured by a large negative contribution from E_{LJ}, and large negative values of E_C are obtained when a hydrogen bond is established between a hydrogen of one ring and an oxygen of the other ring and/or the glycosidic oxygen. From statistical sums over all internal degrees of freedom[16], the free enthalpy can be calculated; the differences between the lowest and all the others are given in Table II.

The difference between ΔG and ΔE_T for a specific minimum arises from differences in zero-point energy and in the vibrational contribution to the free enthalpy between the minimum in question and the lowest minimum.

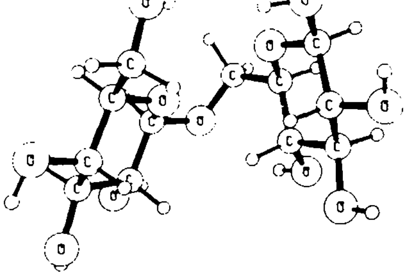
An equilibrium distribution between the lowest minima at 298 K, based on



MINIMUM 5 MINIMUM 5



MINIMUM 9 MINIMUM 9



MINIMUM 7 MINIMUM 7

Fig. 3. ORTEP stereo-drawings of selected, low-energy minimum conformers: (*a*) minimum 5, (ϕ, ψ, ω) = (64, 120, 55); (*b*) minimum 9, (ϕ, ψ, ω) = (106, 86, −37); (*c*) minimum 7, (ϕ, ψ, ω) = (79, 95, 133).

differences in free enthalpy, can be given as minimum 5:minimum 9:minimum 8: minimum 6:minimum 2:all others = 40:20:9:5:5:21. Three low-energy minima are shown in Fig. 3. As pointed out for β-maltose[1] and β-cellobiose[2], each minimum listed in Table II is associated with a manifold of minimum energy conformations corresponding to different values of the exocyclic torsional angles.

The very many ϕ,ψ,ω-minima also explain the difficulty in preparing crystals suitable for crystal-structure analysis[10]. Furthermore, it would be predicted that the barriers between the lowest minima should be lower than those calculated for β-maltose[1] and β-cellobiose[2], since the β-(1→6) linkage reduces the strong repulsion between the two rings and the atoms bonded directly to them.

Comparison with experimental results. — It should be noted that the n.m.r. experiments were performed on peracetylated β-gentiobiose in $CDCl_3$, where hydrogen bonds play a minor role, whereas the calculations were performed on free β-gentiobiose in a hypothetical gas-phase, where intramolecular hydrogen-bonding is prominent. Nevertheless, some general features can be compared. Neither case will necessarily apply in detail to conditions in aqueous solution, where hydrogen-bond structure may be modified appreciably.

Concerning the conformation around ϕ, the n.m.r. results are more or less based on the exo-anomeric effect. Pérez and Marchessault[15] found experimental evidence for this effect from crystal structures. For β-pyranosides and β-linked oligosaccharides, they found a very narrow distribution for ϕ-angles ranging from 10° to 50°. The calculated range for the low-energy conformation is broader.

In some of the low-energy conformations, including the lowest, ω-values around +60° are observed. In these conformations, the unfavourable synperiplanar O-6---O-4 interaction is counterbalanced by a strong, bifurcated hydrogen-bond H(O-4)---O-6 and H(O-4)---O-5′. This situation does not occur for $\omega \simeq$ 180 or 60°, and therefore conformations of the free disaccharides with $\omega \simeq 60°$ are probably over-preferred when only intramolecular interactions are taken into account. This may also be the reason why many of the low-energy conformers show ψ-values around +120°. The weighted averages, using the distribution based on differences in free enthalpy, for the distances H_R(C-6)---H(C-1′) and H_S(C-6)---H(C-1′) are 3.2 Å and 2.8 Å, respectively, in contradiction to the n.m.r. results[11,12] on the non-hydrogen-bonded octa-acetate.

CONCLUSION

In the previous papers of this series, we described how it is possible, given proper minimisation techniques and a reasonable force-field, to calculate realistic structures for such flexible molecules as the disaccharides β-maltose and β-cellobiose.

In this paper, the multidimensional energy-hypersurface for β-gentiobiose is explored by using a refined force-field with inclusion of electrostatic interactions. Many local minima are observed, and the energy differences between the lowest ones indicate that β-gentiobiose is extremely flexible. This might explain the difficulty in obtaining suitable crystals for structure analysis.

Disaccharides show a remarkable difference in conformational flexibility, as testified by the calculations on maltose, cellobiose, and gentiobiose. The α-(1→4)-linked maltose is rather restricted (4 minima), the β-(1→4) linkage in cellobiose gives a little more conformational freedom on a more-complex energy surface (6 minima), and the β-(1→6)-linked gentiobiose, with an extra degree of rotational freedom, shows extreme conformational flexibility (24 minima).

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APPENDIX

α,β-D-Glucopyranose. — There is no significant difference between the prior FF300 and the present FF400, when comparing calculated internal co-ordinates with crystal-structure data. Looking at the ring geometry, the $^4C_1$ conformer is ~10 kJ.mol^{-1} more stable than the $^1C_4$ and the six twist-boat conformers. This is in accordance with experimental evidence. The calculated anomer ratio, based on the difference in free enthalpy, is $\alpha:\beta = 0.34:0.66$, in agreement with experiments.

β-Maltose. — Minimum 5, previously reported[1] as probably a spurious minimum, does not exist in FF400, but is minimised to the new minimum 1*, which is close to the former minimum 3 (FF400-minima are marked with an asterisk). This gives the following FF400-minima, which, on the ϕ,ψ-map, are close to those of the former calculations:

Minimum	(ϕ, ψ)	ΔE_T	ΔG
1*	(66, −50)	0.0	0.0
2*	(18, 31)	3.8	6.1
3*	(3, 33)	6.9	6.8
4*	(−41, −162)	10.9	12.7

The largest change occurred from the previous minimum 2 (17,19) to the present minimum 3*. The equilibrium distribution, based on differences in free enthalpy at 298 K, of minimum 1*:minimum 2*:minimum 3*:minimum 4* = 86:8:5:1 (15:59:19:7, previously reported).

β-cellobiose. — Six local minima are found as before[2], and the (ϕ,ψ)-positions are almost the same but, as for β-maltose, with a different order on the energy scale.

Minimum	(ϕ, ψ)	ΔE_T	ΔG
1*	(61, 3)	0.0	0.0
2*	(162, 5)	1.2	3.4
3*	(72, −167)	10.0	6.9
4*	(29, 62)	11.6	10.3
5*	(48, 166)	11.9	12.6
6*	(170, −143)	25.0	33.2

The equilibrium distribution (298 K) of minimum 1*:minimum 2*:minimum 3*:minimum 4*:minimum 5*:minimum 6* = 75:19:4:1:1:0 (60:3:1:34:2:0, previously reported).

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