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Potential Energy Surfaces of Cellobiose and Maltose in Aqueous Solution: A New Treatment of Disaccharide Optical Rotation

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Abstract: The problem of determining conformational preferences for oligo- and polysaccharides in solution is best approached by establishing, in increasingly greater detail, the features of their potential energy surfaces as functions of the linkage dihedral angles ϕ and ψ. Beginning with a survey of calculated in vacuo potential surfaces for cellobiose and maltose, we examine the results of optical rotation and NMR measurements and develop a picture of their potential surfaces in aqueous solution. In doing so we apply a new semiempirical theory of saccharide optical activity. The results confirm many of the previously emphasized conformational features and indicate the likely role of "folded" conformations in providing stable turn geometries for the cellulose and amylose polymers.

Polysaccharides are the most plentiful biopolymer. In the biosphere there is probably more carbohydrate than all other organic matter combined, largely because of the abundance in the plant world of two polymers of D-glucose, cellulose and starch.[1] The commercial exploitation of polysaccharides is growing,[2] as is awareness of the breadth of their biological function.[3] The progress that has been made in characterizing their structure at the molecular level has come from applying many physicochemical techniques in conjunction with one another.[4-6]

Chiroptical properties are useful[7] because of their great sensitivity to chemical structure, configuration, and conformation, and there has been a systematic improvement in the empirical and semiempirical methods of interpreting experimental data. The very early empirical rules of Hudson[8] have been superseded through the work of Whiffen,[9] Brewster,[10] and others, from which it is now possible to rationalize, or predict, the observed molar rotation of a wide variety of molecules. Those methods are empirical in that all parameters are derived from experimental data, but the analysis that leads to the parameterization has some theoretical basis.[11]

Rees and co-workers[12,13] extended those treatments to include the conformational dependence of polysaccharide optical rotation. A linkage contribution to optical rotation is defined in terms of experimental data, and a procedure developed for its calculation from the ϕ,ψ angles that specify the linkage geometry. The method allows a screening of those conformations that are compatible with the experimental data.

Prompted by features observed in the vacuum ultraviolet circular dichroism (CD) of saccharides, Stevens and Sathyanara-

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yana[14-17] have recently developed a semiempirical theory of saccharide optical rotation. Their method represents a modification of Kirkwood's polarizability theory of optical activity,[18,19] in which the high-energy electronic transitions associated with the valence electrons of all CC, CO, and CH groups of the molecule are coupled through perturbation theory. The theory has been successful in describing the dependence of molar rotation on chemical structure and configuration for a large number of saccharides, ethers, and cyclohexanepolyols.[14-17]

Motivated by the success of those applications, we have here extended the method to disaccharides by including the conformational dependence of molar rotation on linkage geometry and applied it to two prototypic disaccharides, cellobiose and maltose. In relating our results to theoretical energy calculations and other experimental methods, we refer to conformational *types*, representing defined regions of conformation space, rather than specific conformers corresponding to single points in that space. Such an approach is appropriate especially for the solution conformations of cellobiose and maltose, where the important regions of the energy surfaces contain relatively shallow minima separated by low barriers and saddle points.

Calculational Methods

The method we use to calculate Na_D molar rotation, $[M]$, has previously been described in detail,[14] and its applicability to pyranoses, pyranosides, and other model compounds demonstrated.[15-17] The model is essentially one of coupled oscillators and requires the solution of the secular equation

$$\sum_{i=1}^{N} C_{ik}(V_{ij} - E_k\delta_{ij}) = 0 \qquad j = 1, 2, ..., N$$

for the eigenvalues E_k and coefficients C_{ik}. The V_{ij} matrix elements represent the Coulombic interactions between the unperturbed electronic transition moments μ_i and μ_j, localized on the CC, CH, and CO bonds of the molecule. Three orthogonal transition moments are placed on each of the n bonds, so that there are $N = 3n$ values of E_k. The C_{ik} describe the interacted (molecular) transition moments as linear combinations of unperturbed (bond) moments. Rotational strengths, CD, and $[M]$ are then calculated by well-known equations.[20,21] The parameterization originally optimized for saccharide fragments[14] (e.g., CH_2OH–CH_2OH) was used unmodified for the present work.

The calculated molar rotations refer to molecules in a vacuum. For comparison with solution data a solvent correction can be applied[21] in which all calculated rotations are multiplied by $(n^2 + 2)/3$, where n is the refractive index of the solvent. For water the solvent correction term is 1.26.

The results previously reported for saccharides, pyranosides, cyclic ethers, and other carbohydrate model compounds[16,17] were successful in reproducing the observed dependence of molar rotation on chemical structure, but the calculated in vacuo values of $[M]$ were too small by a factor of $\sim$2, part of which can be accounted for by the solvent correction. For the present work we incorporated into the method an empirical scale factor that brings the calculated molar rotations of the monomeric compounds into almost quantitative agreement with experimental data. Although empirical in nature, there are theoretical grounds for believing that it represents those contributions to optical activity omitted in the Kirkwood theory (see Discussion).

Table I. Calculated and Observed Molar Rotations (deg cm^2 dmol^{-1}) for Six Hexopyranosides

compd	$[M]^a$	$[M]^b$	$[M]^c$	$[M]^{obsd\ d}$
methyl β-D-mannopyranoside	−137	−135	−118	−136
methyl β-D-glucopyranoside	−68	−75	−60	−66
methyl β-D-galactopyranoside	−3	−25	29	0
methyl α-D-mannopyranoside	144	130	189	154
methyl α-D-glucopyranoside	309	325	289	309
methyl α-D-galactopyranoside	374	375	392	380

[a] Calculated by Whiffen's method.[9] [b] Calculated by Brewster's method.[10] [c] Present work. [d] Experimental value.[23]

We determined the scale factor using the calculated and observed molar rotations of six hexopyranosides that best approximate the structural elements of the disaccharides of interest, the α- and β-methyl pyranosides of mannose, glucose, and galactose. For each of the six compounds $[M]$ was previously calculated[17] for the two predominant hydroxymethyl conformers, gg and gt for glucose and mannose pyranosides and gt and tg for galactose pyranoside. (The notation specifies the orientation of the C(6)–O(6) bond, gauche or trans, relative to the C(5)–O(5) bond (first letter) and to the C(5)–C(4) bond (second letter).) The calculated values for each conformer[17] were solvent corrected and then combined to give a calculated weighted average for each pyranoside, using the estimated weights proposed by Lemieux and Brewer,[22] as follows:

$$[\bar{M}] = 0.67[M]_{gt} + 0.33[M]_{gg}$$

for the glucose and mannose compounds, and

$$[\bar{M}] = 0.50[M]_{gt} + 0.50[M]_{tg}$$

for galactose compounds. A least-squares fit of the six calculated values to the corresponding observed values was determined, constraining the fit to pass through the origin. The slope of the fit was 0.59, and the scale factor was taken to be its inverse, 1.69. Application of this scale factor to the solvent-adjusted calculated values reproduces the observed molar rotations to within ±24 deg cm^2 dmol^{-1}. The results are summarized in Table I and compared with the empirical methods of Whiffen[9] and Brewster.[10]

Disaccharide calculations were then carried out on β-cellobiose and β-maltose. Coordinates for the D-glucose rings in the $^4C_1$ conformation were adapted from Arnott and Scott.[24] Hydroxyl hydrogen atoms do not enter into the calculation; their coordinates need not be specified. The hydroxymethyl groups were both placed either in the gg or gt conformation (see below).

Within the glycosidic linkage, β in cellobiose and α in maltose, the C–O–C bond angle was taken to be 117.5°.[25-27] The linkage conformation is then specified by the angles ϕ and ψ, where $\phi = 0°$ corresponds to the C(1)–H bond cis to the O(1)–C′(4) bond, and $\psi = 0°$ corresponds to the O(1)–C(1) bond cis to the C′(4)–H bond. Positive values of ϕ and ψ refer to clockwise rotation of the reducing residue as viewed from the nonreducing residue.

For cellobiose, the conformations that were examined (Table II) span the region of (ϕ,ψ) space believed to include all of the energetically preferred conformations. They include the X-ray-determined structures of cellobiose (X1, X2)[25,26] and cellobioside (X3)[28] and the results of empirical potential energy calculations using a variety of functional forms and parameterizations (RS1, YR, SR, RS2, LVK, LSK),[29-33,37] PCILO calculations (GPM),[34]

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Table II. Calculated Molar Rotation (deg cm^2 dmol^{-1}) of Cellobiose as a Fundtion of Conformation ($[M]^{obsd}$ = 24.3)

conf type	ref[a]	(ϕ,ψ)	$[M]^{calcd}_{gg,gg}$	$[M]^{calcd}_{gt,gt}$	$[\bar{M}]^{calcd}$
A	GPM	98,-24	-14	67	40
	GPM	94,-22	-14	67	40
	YR	60,20	-16	49	27
	LSK	60,10	-13	57	34
	MR2	61,3	-11	60	36
	LVK	60,0	-12	62	37
	LVK	55,20	-14	49	28
	MR1	51,0	-10	60	37
AB	RS1	41,-5	-10	56	34
	X1	44,-12	-15	53	30
	X2	42,-18	-20	53	29
	SR	40,-20	-22	45	23
B	RS2	30,-25	-27	32	12
	LVK, LSK	30,-40	-46	9	-9
	LVK	25,-35	-38	16	-2
	X3	25,-48	-54	-5	-21
	GPM	23,-44	-48	0	-16
	MR2	29,-62	-78	-27	-44
C	RS1	0,-37	-24	8	-3
	MR1	-10,-29	-12	17	7
	LSK	-20,-20	-1	23	15
	LVK	-20,-25	-4	20	12
D	SR	170,0	-1	52	35
	YR	170,15	-9	41	24
	MR1	164,5	-8	54	33
	MR2	162,5	-10	46	27
X	MR1	21,172	-138	-93	-108
	LVK	30,175	-148	-93	-111
	YR, LSK	30,180	-148	-94	-112
	MR2	48,166	-156	-90	-112
Y	MR1	67,203	-184	-117	-139
	GPM	78,186	-185	-116	-139
	MR2	72,193	-184	-117	-139
Z	MR2	170,217	-136	-55	-82
	MR1	181,205	-140	-64	-89

[a] GPM,[34] YR,[30] LVK,[33] MR1,[35] MR2,[36] RS1,[29] X1,[25] X2,[26] SR,[31] RS2,[32] X3,[28] LSK.[37] One GPM conformer, (41,-84), was omitted from the table; it falls outside the space defined by all other calculations.[34]

and empirical force-field calculations (MR1, MR2).[35,36]

For maltose (Table III) the conformations examined similarly span the energetically favorable region of (ϕ,ψ) space, including X-ray structures (X1, X2)[27,38] and the results of potential energy calculations (RS, LVK, BSM, SR, SG, RYS, RSRR, B, SLK),[32,33,39-45] PCILO calculations (GPM),[34] and empirical force-field calculations (MR2, MR3).[36,46]

Table III. Calculated Molar Rotation (deg cm^2 dmol^{-1}) of Maltose as a Function of Conformation ($[M]^{obsd}$ = 201)

conf type	ref[a]	(ϕ,ψ)	$[M]^{calcd}_{gg,gg}$	$[M]^{calcd}_{gt,gt}$	$[\bar{M}]^{calcd}$
A	SG	70,20	36	20	25
	SG	50,40	49	41	44
	LVK	30,25	73	70	71
	SLK	20,30	85	84	84
	MR3	20,18	90	88	89
	SR, BSM, SLK	20,20	89	88	88
	SG	0,50	102	113	109
AB	SG	10,20	103	103	103
	MR2	3,33	106	113	111
	X1	3,10	113	112	112
	SG	0,0	116	112	113
	X2	-9,9	130	128	129
	GPM	-12,-5	133	127	129
	SR	-10,30	126	114	118
B	LVK	-20,-10	143	134	137
	BSM	-20,-15	146	137	140
	B, SLK	-20,-20	146	136	139
	MR2	-18,-31	142	131	135
	MR3	-22,-23	150	140	143
	RSRR	-30,-10	159	149	152
	RYS,SLK	-30,-20	161	150	154
	RS	-35,-15	169	159	162
	GPM	-33,-22	167	156	160
	SG	-40,0	170	161	164
	GPM	-42,-9	178	166	170
C	MR2	-66,-50	231	230	230
	MR3	-66,-43	228	223	224
	LVK	-70,-35	228	216	220
	RS, SLK	-70,-40	231	227	228
	SR	-70,-60	237	242	240
	BSM	-80,-45	244	241	242
X	MR3	-30,-169	119	135	130
	MR2	-41,-162	136	152	147
	B	-40,-160	137	153	148
	LVK, SLK	-30,-160	120	126	124

[a] SG,[41] LVK,[33] MR2,[36] MR3,[46] SR,[40] BSM,[39] X1,[38] X2,[27] GPM,[34] B,[44] RSRR,[43] RYS,[42] RS,[32] SLK.[45]

Calculated results were solvent corrected and the 1.69 scaling factor applied to give $[M]^{calcd}_{gg,gg}$ and $[M]^{calcd}_{gt,gt}$. If the statistical weights of the hydroxymethyl group conformations in D-glucose (above) are retained in the disaccharide, the weighted average we require is given by

$$[\bar{M}]^{calcd} = 0.45[M]^{calcd}_{gt,gt} + 0.22[M]^{calcd}_{gt,gg} + 0.22[M]^{calcd}_{gg,gt} + 0.11[M]^{calcd}_{gg,gg}$$

where $0.45 = (0.67)^2$, $0.22 = (0.67)(0.33)$, and $0.11 = (0.33)^2$. We assume that the contribution of each disaccharide hydroxymethyl group to optical rotation is independent of the conformation of the other, so that

$$[M]_{gt,gg} + [M]_{gg,gt} = [M]_{gg,gg} + [M]_{gt,gt}$$

In that case the required average simplifies to

$$[\bar{M}]^{calcd} = 0.67[M]^{calcd}_{gt,gt} + 0.33[M]^{calcd}_{gg,gg}$$

Results

As described previously,[14-17] the theoretical model we have applied consistently results in one CD band appearing in the spectrum at a wavelength well separated from all other higher energy bands. In the present case that band is near 167 nm. Its rotational strength depends on the geometry of the molecule and largely determines the Na_D molar rotation.

Tables II and III show the calculated rotations for cellobiose and maltose, respectively. The values shown are on a residue basis, in units of deg cm^2 dmol^{-1}. The observed rotations, per residue, are +24.3 deg cm^2 dmol^{-1} for β-cellobiose and +201 deg cm^2 dmol^{-1} for β-maltose.[27] In the Discussion units are omitted for brevity.

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Discussion

Calculational Method. The assumptions and approximations in the calculational method have been discussed previously in detail.[14] A fundamental approximation is the use of Kirkwood's polarizability theory of optical activity.[18,19] In that theory the molecular optical activity is decomposed into contributions from component groups, and the phase of the incident light is taken to be equal at every point in a given group. Our model includes the coupling retained by that approximation, the μ–μ coupling. Omitted is the μ–m coupling between electric-dipole transition moments and intrinsic magnetic-dipole transition moments.[47] In a recent ab initio calculation of the optical activity of methyl derivatives of cyclopropane, Bohan and Bouman[48] found the μ–μ coupling to make, consistently, the largest contribution to rotational strength, but the μ–m coupling contribution, in the cases examined, were of the same sign and order of magnitude. An intriguing possibility is that, for reasons not yet clear, their result applies more generally to saturated hydrocarbons and, perhaps, to saturated oxo compounds, i.e., to all cases dominated by σ–σ^* electronic transitions. The fact that the previous applications of the present model gave results that were systematically too low[14,16,17] is circumstantial support for such a suggestion. The scaling that we introduce here may eventually be found to have some theoretical justification as a representation of the μ–m coupling contributions omitted in our formalism. At the present time, however, it must be considered simply an empirical adjustment.

The comparison of calculated Na_D molar rotations with observed values (Table I) is relatively good; the standard deviation is ±24 deg cm^2 dmol^{-1}. The calculated weighted average over hydroxymethyl group conformers was obtained by using Lemieux and Brewer's proposed statistical weights.[22] It would only take slight variations of those weights to bring the calculated and observed values into exact correspondence (Table I), but the approximate nature of the theory does not justify such an adjustment.

In interpreting our calculated optical rotation dependence on torsional angles in disaccharides we are therefore justified in attributing significance only to optical rotation differences several times larger than ±24 deg cm^2 dmol^{-1}. Our major conclusions will be seen (below) to depend on large calculated differences.

Cellobiose. The calculated optical rotation is sensitive to the conformation of the exocyclic hydroxymethyl group (Table II), as expected. The difference in calculated rotation between the gg and gt conformers is approximately +65 for most linkage conformations, which is significantly close to the value Lemieux and Brewer[22] estimate it to be using empirically derived fragment parameters. This agreement provides an illustration of the correspondence between the present calculational method and the earlier empirical treatments.[9,10] It also supports the notion that non-nearest-neighbor interactions do not affect the dependence of optical rotation on hydroxymethyl conformation in β-anomers and thereby lends credence to our method of averaging over hydroxymethyl group conformers.[22]

The conformations we have examined (Table II) span the relevant (ϕ,ψ) space (see above). Except for the X-ray structures, they all appear as local minima in various calculated potential surfaces. The potential surface in vacuo consists of a high-energy barrier separating two broad regions[35,36] defined approximately as ψ = 0° (A–D) and ψ = 180° (X–Z). Within each of these two regions the barriers are relatively small. All workers agree that the conformations in the first region are energetically favored over those in the second; they include, for example, the solid-state X-ray-determined structures. Workers do not agree on the detailed shape of the potential within the region A–C. Some find three distinct minima, others only two or one.

Lipkind et al.[33] found the potential surface in the region A–C to be particularly sensitive to the choice of potential function, as did Melberg and Rasmussen in two successive force-field calculations.[35,36] Taken together, however, the theoretical work provides a consistent picture of a rather flat potential surface in the region A–C, in which the molecule is relatively free to move about.

Because of the apparent sensitivity of the potential surface to parameterization and because solvent was included in none of the energy calculations, we made no attempt to calculate a weighted average of optical rotation over (ϕ,ψ) values. Nevertheless, we can draw conclusions of a qualitative nature, and thereby derive a qualitative description of the potential surface of cellobiose in aqueous solution, by comparing the results of Table II with the observed rotation in water, +24.3.

The first conclusion based on our calculations of optical rotation is that conformations in the region X–Z, in which ψ is approximately 180°, are of small importance. The optical rotations calculated for that region are 100–160 deg cm^2 dmol^{-1} more negative than the observed rotation, a difference that is several times the level of uncertainty in the method (see above). Most in vacuo energy calculations lead to the same conclusion. Force-field calculations lead to a combined statistical weight of 3–5% for that region of (ϕ,ψ) space.[35,36]

On the other hand, the observed rotation is what is calculated for the X-ray structure, X2, and fluctuations about the X-ray structure tend to have opposing and canceling effects on rotation; i.e., positive increments in ϕ or ψ lead to positive changes in the calculated rotation, and negative changes in ϕ or ψ lead to the opposite. The observed optical rotation is therefore consistent with energy calculations that depict a rather flat potential surface in the region A–C. Conformational excursions within that region may be rather large. The lack of any temperature dependence of optical rotation in water over the range 20–80 °C[13] is also a sign of a relatively flat potential surface.

Our results further indicate that optical rotation cannot distinguish a cellobiose potential surface in which AB, representing the X-ray structure, is a minimum from a surface in which AB is a saddle point separating regions A and B. The more recent energy calculations[33,35,36] indicate it is a saddle point, but one that provides only a shallow barrier between A and B.

A common structural feature of all conformations in the region A–B, largely obscured by the wide variation in (ϕ,ψ) angles, is a nearly constant O(5)–O′(3) distance. This feature is apparently the result of nonbonded interactions inasmuch as the region is favored whether or not hydrogen-bonding terms are included in the potential function.[30,31,33] The fluctuations within this region may therefore be pictured as occurring within the context of maintaining that favorable oxygen–oxygen interaction.

The calculation is indeterminate with respect to region C; the calculated optical rotation in that region is not significantly different from the observed value. From energy calculations alone it is not even clear whether that region is merely an extension of the B region or is separated from it by another saddle point. Lipkind et al.[33,37] found it to be a separate minimum but with only marginal statistical weight (6–10%). Melberg and Rasmussen found a minimum in region C in an early force-field calculation (MR1),[35] which was replaced with a minimum in region B (MR2)[36] upon refinement of the potential function. Thus, neither energy calculations nor optical rotation analysis is sufficiently refined at this stage to clarify this point.

The present work indicates that conformations in region D may be present to a significant extent; the calculated rotation for that region is also close to the observed value. Region D has been identified by only a few energy calculations[30,31,35,36] but those indicate that the barrier between it and the major A–C region is not large. Successive force-field calculations[36,37] assign it a statistical weight of 3–19% in vacuo. The conformation is of particular interest because of its potential importance in providing stable turn conformations for the cellulose polymer.[31] The present work indicates that region D should not be overlooked in future energy calculations.

Rees previously concluded,[12,13] on the basis of a linkage rotation analysis, that the observed optical rotation of cellobiose is what is expected for the X-ray structure, as we do here. The details of the two methods are different, however, which results in a somewhat different (ϕ,ψ) dependence of rotation in the region

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around the X-ray structure. At the present time it would be difficult to establish which method gives a more accurate description of the details of this dependence, but it is satisfying that both methods of interpreting optical rotation lead to the same general qualitative conclusion.

A variety of NMR techniques have been applied to the determination of cellobiose conformation in water. If the potential energy surface within the allowed regions is relatively flat and the barriers between populated regions small, one expects the rates of conformational interchange to be fast relative to the NMR time scale, and this expectation is confirmed, at least for the region A–C, by an estimate of those rates based on force-field calculations.[35] Therefore, NMR, like optical rotation, reflects a weighted average conformation.

Early measurements of ^{13}C chemical shifts[49] showed only that there are no strong intramolecular steric interactions between the two rings. Later NMR studies were aimed at determining whether the average solution conformation was compatible with the X-ray-determined structure. Thus, Hamer et al.[50] measured ^{13}C–^{1}H coupling constants for the two ^{13}C–O–C–^{1}H arrays specified by ϕ and ψ and, revising earlier estimates[51] upward, reported values of 4.2 Hz for $J(\phi)$ and 4.3 Hz for $J(\psi)$. From parameterization of a Karplus relationship they found that the observed coupling constants are compatible with average ϕ and ψ values of $\pm(25–30°)$ or $\pm(145–150°)$. The 16 (ϕ,ψ) combinations thus allowed by the degeneracy of the Karplus relationship include only two compatible with calculated potential surfaces, i.e., approximately (+30°, −30°) and (−30°, −30°) (Table II). Taking account of the uncertainty in the experimental measurement, one can conclude from the coupling constant data only that the broad region A–C is the most populated region.

As the potential surface came to be determined in more detail, results of NMR measurements have been analyzed with the aim of estimating the relative populations of specific calculated local minima. For example, proton relaxation measurements (cited in ref 35) give an average value for the C(1)H–C′(4)H separation of 2.1–2.2 Å. The separation for conformations in the region A–C is 2.2–2.3 Å; for conformations D and X–Z it is 3.5–4.0 Å. Thus, that experiment also indicates the importance of the region A–C. A calculated statistical weighting of 3% for D is also compatible with the observed average value;[35] but the statistical weighting of 19% for D reported later[36] gives a value of 2.5 Å, which may indicate that weighting is too high.

Lipkind et al.[37] measured nuclear Overhauser enhancements resulting from preirradiation of C(1)H and compared the experimental results with calculated enhancements for conformations A, B, C, and X (D was not included). A strong enhancement of the C′(4)H resonance indicates a large population of region A–C, in agreement with previous work. A positive enhancement of the C′(6)H resonance indicates, in particular, a large population of region A, where the large positive value of $\phi + \psi$ brings the C′(6) hydroxymethyl group close to C(1)H. A small positive enhancement of the C′(3)H and C′(5)H resonances was taken to indicate a significant population (10% or more) of conformers in the X region, because only in that region, among the four considered, are positive enhancements expected by virtue of ψ being nearly 180°. However, a conformer in region D, not considered in that analysis, would give a similar result because of its large value of ϕ. The force-field calculation[25] indicates region D to be significantly preferred over X, and additional evidence for a significant presence of D conformers, but not X, comes from the ^{13}C–^{1}H coupling constant data. Conformers in the A region, certainly present to a large extent, have a small $J(\phi)$ coupling constant (~2 Hz) because of the large value of ϕ (60°), yet the observed value is 4.2 Hz. The presence of a conformer with a large $J(\phi)$ coupling constant would tend to compensate, and the D conformer, with a ϕ value near 180°, meets the requirement, but not the X conformer. The NOE experiment thus provides evidence of a largely populated A–C region, with a significant but small population of the D region. It is thereby in accordance with optical rotation data.

In summary, the combined calculated in vacuo potential energy surfaces, together with experimental optical rotation and NMR data for aqueous solution, provide a rather consistent and detailed picture of the potential energy surface of cellobiose in water. The regions A–C and D, separated by a small barrier, account for almost the entire populated region, not less than 95%. Region D can be estimated to account for approximately 10%, and the region A–C the remaining 85%. There is probably a saddle point near the A–B region separating two approximately equally populated regions, one of which is A and the other the combined B, C region.

Further details of the potential surface may require extensive computer simulations that include the solvent. The height of the barrier separating the A–C and D regions will be of interest and may prove to be particularly important for understanding the properties of cellulose polymers.

Maltose. In α-linked disaccharides the optical rotation is significantly affected by an interaction between the axial C(1)–O bond and the hydroxymethyl group C(6)–O bond when that group is in the gt conformation. The negative contribution to optical rotation that results combines with the positive increment that results from the gg–gt conversion (see above). The net effect is that the calculated rotation is almost the same for gg and gt conformations (Table III), illustrating the role non-nearest-neighbor interactions can have on optical rotation.

The linkage conformations we have examined (Table III) include all those that have appeared as local energy minima in various calculated in vacuo potential energy surfaces. The potential surface consists of a high barrier separating two regions defined approximately as $\psi = 0°$ (A–C) and $\psi = 180°$ (X).[46] (Regions corresponding to D, Y, and Z of the cellobiose surface are precluded by the α-linkage in maltose.) The broad region A–C is energetically favored over region X, so that it accounts for almost all of the populated (ϕ,ψ) space.[46] Taken together, the calculated in vacuo potential energy calculations depict a relatively flat energy surface in the region A–C with three shallow minima. Regions A and B are separated by a saddle point region A–B containing the X-ray structure, which is similar to the case of cellobiose (see above). A slightly larger barrier separates regions B and C.

Regions A and B are characterized by a favorable O(2)–O′(3) separation, which is replaced in region C by a favorable O(5)–O′(3) separation, the latter being the same as was found to be important in cellobiose (see above).

The detailed features of the calculated surfaces are extremely sensitive to the form and parameterization of the potential function. In two successive force-field calculations[36,46] the statistical weight of region C increased from 15% to 87%, while that of region B decreased from 59% to 8%. Changing ring atom coordinates from those of methyl β-maltose determined from X-ray diffraction to those of methyl α-maltose determined from neutron diffraction caused an increase in the statistical weight of region C from 40% to 60% and a decrease of region B from 45% to 30%.[45] These results indicate the relative flatness of the potential surface in the region A–C and that experimental studies should be sought to elaborate its details in aqueous solution.

Several conclusions can be drawn from the present calculations (Table III). First, consistent with the most recent in vacuo calculations, region C must also be the most populated in aqueous solution in order to account for the large positive optical rotation observed (+201). Regions B and X display calculated optical rotations that are 50–100 deg cm^2 dmol^{-1} more negative than the observed value and therefore must be populated to a lesser extent. Region A with its smaller calculated optical rotation is likely populated only slightly.

Rees, on the basis of linkage rotation analysis,[12,13] similarly concluded that the average conformation in aqueous solution is better represented by region C than by the X-ray structure. This departure from the solid-state structure upon dissolution indicates

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that the O(2)–O′(3) hydrogen bond of the solid-state structure is broken. The favorable O′(3)–O(5) interaction that replaces that hydrogen bond does not itself require hydrogen bond formation (see cellobiose above). The shift to lower optical rotation in less polar solvents is consistent with such a description.[12,13]

Results of NMR experiments also provide support for this picture. Dorman and Roberts observed ^{13}C chemical shift evidence for intramolecular steric interactions in maltose, in contrast to cellobiose (see above).[49] Specifically, the shift in the C′(3) resonance is donwfield of the corresponding resonance in methyl β-D-glucoside. They proposed a conformation in which C′(3) is brought into proximity with C(2) and C(3), as in region A, but the C-type conformation would provide a similar perturbation.

NOE enhancements[45] arising from preirradiation of C(1)H are greatest for the C′(4)H resonance, showing the effect of regions A and/or B. In region C a small enhancement is expected for both the C′(3)H and C′(4)H resonances, and the small enhancement observed in the C′(3)H resonance[45] reflects its significant population.

^{13}C–^{1}H coupling constants have been measured for methyl β-maltoside.[45,52] $J(\psi)$ can be obtained directly from a gated-decoupling spectrum, and the recent measurement of 3.8 Hz[45] is consistent with a preponderance of the C-type conformation. $J(\phi)$ can only be estimated from the separation between extreme multiplet components with and without preirradiation of C(1)H, giving a result of 3.5 ± 0.3 Hz.[45] Conformers in region C, because of the large value of ϕ, would contribute 1–2 Hz, so that a predominance of the C-type conformation is consistent only with the lower value in the reported range.

Tvaroska[53] modified the in vacuo potential energy surface of Melberg and Rasmussen[46] to incorporate solvation, the major effect of which was to stabilize region X substantially so that it became the most populated. The optical rotation data, as analyzed here and previously, are not consistent with that result. Applying Tvaroska's calculated free energies of solvation to other, more recently calculated, in vacuo potential surfaces[36,45] does not eliminate this difficulty, which can be traced to the particularly large electrostatic contribution to solvation free energy calculated for the X region.[53] Perhaps it will be found necessary to apply direct large-scale computer simulations to incorporate solvent into energy calculations realistically.

In summary, the optical rotation and NMR data give a consistent picture of some of the detailed features of the potential energy surface for maltose in aqueous solution. Of the reported in vacuo potential surfaces, that calculated by Shashkov et al.[45] for methyl α-maltoside using glucose coordinates derived from neutron diffraction data most nearly matches the features derived here. The two successive force-field calculations give lower and higher populations, respectively, for region C.[46,36]

Finally, we wish to note that although our present results indicate the preponderance of the C-type conformation for maltose in aqueous solution, which is in agreement with the results of Shashkov et al.,[45] we do not agree with those workers' interpretation of their results[33,45] as demonstrating the absence of an exo-anomeric effect in contributing to the conformational preferences of oligosaccharides. Such an effect has been proposed as contributing to the dependence of torsional energy on rotation about the glycosidic bond, ϕ.[54-56] The comparison Shashkov et al. made was of (a) a Scott–Scheraga nonbonded potential supplemented with a conventional torsional term having a barrier height of 0.9 kcal mol^{-1} with (b) an HSEA (hard-sphere exo-anomeric) calculation, i.e., a Kitaigorodski nonbonded potential supplemented with an exo-anomeric torsional term having a barrier height of 1.72 kcal mol^{-1}. Two reasons make us believe the difference in global minimum in the two calculations arises more from the difference in nonbonded energy terms than from the different torsional contributions. First, with a Kitaigorodski nonbonded potential and conventional torsional term no energy minimum appears for the C-type conformation (Figure 5 of ref 33); that conformation is a global minimum only when the Scott–Scheraga nonbonded potential is used.[33,45] Secondly, the exo-anomeric torsional term has a *greater* preference for values of ϕ near −60° than does the conventional torsional function, making it likely that the C-type conformation is missed in an HSEA calculation on account of its use of a Kitaigorodski nonbonded potential. In our view, which form and parameterization of torsional contribution is most appropriate for oligosaccharides is still an open question, as is the question of what part of that contribution is appropriately considered as arising from an exo-anomeric effect.

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Registry No. Cellobiose, 528-50-7; maltose, 69-79-4; cellulose, 9004-34-6; amylose, 9005-82-7; methyl β-D-mannopyranoside, 22277-65-2; methyl β-D-glucopyranoside, 709-50-2; methyl β-D-galactopyranoside, 1824-94-8; methyl α-D-mannopyranoside, 617-04-9; methyl α-D-glucopyranoside, 97-30-3; methyl α-D-galactopyranoside, 3396-99-4.

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