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Note

Lactose conformation in aqueous solution from optical rotation

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A newly developed calculational model for saccharide optical rotation[1–4] has recently been extended to disaccharides, with applications to cellobiose and maltose[5]. Here we apply the model to β-lactose [β-D-galactopyranosyl-(1→4)-β-D-glucopyranose]. The purpose in doing so is to demonstrate that the model can account for the large difference in optical activity between two disaccharides, lactose and cellobiose, having the same chemical structure in the linkage region, and to develop a picture of the conformational variations of lactose in aqueous solution compatible with calculated potential-energy surfaces,[6,7] n.m.r. studies,[8,9] and measured optical rotation[10].

The calculational method, previously described in detail[1–5], is based on a coupled-oscillator model which requires the solution of the secular equations:

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

where V_{ij} is the coulombic interaction energy of transition dipole moments μ_i and μ_j representing far-u.v. electronic transitions localized on C-C, C-O, and C-H bonds; the model is thereby geometry-dependent. The solution yields eigenvalues, E_k, specifying molecular transition-energies, and eigencoefficients, C_{ik}, which describe the molecular transition-moments as linear combinations of the unperturbed-bond transition-moments. Circular dichroic rotational strengths are obtained directly, from which the optical rotation is calculated via a Kronig–Kramers transform. We report results as the molar rotation [M] at 589 nm on a disaccharide basis.

Atomic coordinates for each residue were adapted from Arnott and Scott[11]. The hydroxymethyl group was placed in either the *tg* or *gt* conformation for galactose, and in the *gt* or *gg* conformation for glucose; that is, for each linkage geometry examined, all four combinations of the exocyclic group conformations were taken into account. The linkage C-O-C bond angle was fixed at 117.5° and linkage conformations expressed as a function of the dihedral angles φ and ψ. The results were solvent-corrected and scaled[5] to give the values displayed in Table I.

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TABLE I

Calculated molar rotation (deg cm^2 dmol^{-1}) of lactose as a function of conformation ([M]^{obsd} = 120)

(φ,ψ)	$[M]_{tg,gg}$	$[M]_{tg,gt}$	$[M]_{gt,gg}$	$[M]_{gt,gt}$	$[\bar{M}]^a$	$[\bar{M}]^b$
40,20	104	122	146	158	134	138
60,20	81	98	133	145	116	122
80,20	56	78	121	121	95	102
40,0	98	117	144	160	132	137
60,0	91	104	151	160	127	135
80,0	78	89	151	155	119	129
−40, −20	134	163	145	174	153	156
−20, −20	94	141	99	147	127	121
0, −20	68	119	77	129	106	100
20, −20	59	100	83	123	97	95
40, −20	60	86	107	129	99	102
−40, −40	146	166	152	173	162	160
−20, −40	95	124	95	124	113	109
0, −40	47	86	49	89	74	68
20, −40	15	53	30	67	47	43

[a] Statistical weights from ref. 12. [b] Statistical weights from refs. 13 and 14.

Averaging the calculated rotation over exocyclic-group conformations requires the assignment of statistical weights to each of the four conformers. In the past[5] we have used statistical weights of 50% *gt* and 50% *tg* for the galactose residue, and 66.7% *gt* and 33.3% *gg* for the glucose residue.[12] Recent n.m.r. studies have led to the assignment of the *gt* galactose rotamer as the predominant one[13], at approximately 65%; and the *gt*:*gg* ratio in glucose as[14] 50:50. We show the conformational averaging by using both sets of statistical weights in Table I. In either case, we assume that those statistical weights are retained in the disaccharide, so that the weighted average required is given by: $[\bar{M}] = w_{1a}w_{2a}[M]_{tg,gt} + w_{1b}w_{2a}[M]_{gt,gt} + w_{1a}w_{2b}[M]_{tg,gg} + w_{1b}w_{2b}[M]_{gt,gg}$ *where* w_{1a} and w_{1b} are the galactose *tg* and *gt* statistical weights, respectively, and w_{2a} and w_{2b} are the respective *gt* and *gg* glucose statistical weights, all on a fractional basis. Table I shows that the averaged molar rotations for the two sets of statistical weights are not substantially different; the observed dependence is not strong enough to affect our conclusions (see later). In Fig. 1, the calculated values based on n.m.r. statistical weights are superimposed on a hard-sphere conformational φ,ψ-map, generated with the atom–atom contact-distances of Rees and Scott[15].

The hard-sphere conformational maps of β-lactose and β-cellobiose are identical because of structural equivalence in the linkage region. The conformational dependence of optical rotation within the large allowed region (Fig. 1), corresponding to extended conformations, is very similar to the conformational dependence of the optical rotation of the cellobiose[5], the optical rotation of β-lactose being uniformly more positive by approximately 100 deg.cm^2 dmol^{-1} as a consequence of epimerization at C-4 and, secondarily, the difference in conformational preference of the exocyclic hydroxymethyl group on the nonreducing residue. The interactions in the folded conformations,

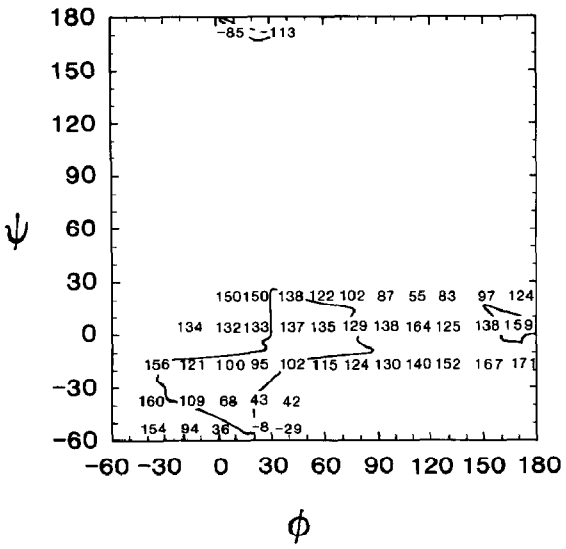

Fig. 1. Na_D molar rotations of β-lactose calculated as a function of linkage angles φ and ψ, superimposed on a hard-sphere conformational map.

represented as the smaller allowed regions in Fig. 1, are more complex, but the overall results are the same as in cellobiose. In the region near $\varphi,\psi = 180°, 0°$ the calculated rotations are similar to those in the major allowed region; near $\varphi,\psi = 0°, 180°$ the calculated rotations are significantly more negative.

The practical usefulness of the calculational model is to elaborate the potential-energy surface of disaccharides in solution, in conjunction with molecular-modeling methods and other experimental techniques, especially n.m.r. The observed optical rotation reflects a weighted average over all populated conformations, as do n.m.r.-derived parameters. In the present application, the observed value[10] of +120 deg cm^2 $dmol^{-1}$, together with Fig. 1, lead to the conclusions that (*a*) the minor allowed region near $\varphi,\psi = 0°, 180°$, with its large negative rotations, is not significantly populated, (*b*) the major allowed region includes conformations having optical rotations near the observed value, and (*c*) the secondary region near $\varphi,\psi = 180°, 0°$ includes conformations having rotations near the observed value, and therefore cannot be excluded from consideration. The major allowed region consists of two domains (Fig. 1), and some calculated potential-energy surfaces[6] indicate that the two are separated, as in cellobiose,[5] by a saddle-point region, namely, a slight energy barrier. Which of the two domains within the region is calculated to be the more stable depends on the form and parameterization of the potential function used.

The question of conformational flexibility in disaccharides, especially in β-linked disaccharides, is at present controversial, with proponents both of conformational flexibility and rigidity. N.m.r. parameters often cannot establish the degree of flexibility because they, like optical rotation, are simple scalar quantities reflecting weighted-average conformations.

Including an energy term for the exoanomeric effect in potential-energy functions for lactose leads to a strong favoring of the $\varphi,\psi = 50°, 10°$ domain of the major region (Fig. 1) and a picture of inflexibility[6]. Nunez and Barker[8] and Hayes *et al.*[9] concluded that the conformation is relatively rigid with respect to rotation about ψ on the basis of their observed $^3J_{C\text{-}1,C\text{-}3'}$ value of 0 Hz, arguing that any significant conformational averaging about ψ would increase the observed value above its minimum possible value. The conformation they derived, however, is $\varphi,\psi = 30°, -15°$, corresponding to the interdomain region. (The value for ψ reported in ref. 9 is $+15°$, but in order for $3'J_{C\text{-}1,C\text{-}3'}$, to be less than $^3J_{C\text{-}1,C\text{-}5'}$ the sign of ψ must be negative.)

Lipkind *et al.*,[7] on the other hand, not including an exoanomeric term in the potential-energy function, found an almost equal weighting of the $\varphi,\psi = 55°, 20°$ domain and the interdomain region of $\varphi,\psi = 30°, -40°$, with small populations of the $\varphi,\psi = -20°, -25°$ domain and the $\varphi,\psi = 30°, 175°$ region; they did not consider the $\varphi,\psi = 180°, 0°$ region. They thereby present a picture of substantial flexibility.

It is possible to combine these three views to generate a picture of β-lactose conformations in aqueous solution as varying over the limited range of φ,ψ values (Fig. 1) from approximately $\varphi,\psi = -30°, -35°$ to $\varphi,\psi = 80°, 5°$. Such a description reflects a conformationally averaged linkage-geometry of approximately $\varphi,\psi = 25°, -15°$, which is consistent with the energy calculations[6,7] and n.m.r. studies,[8,9] and also reflects a greater inflexibility in rotation about ψ relative to φ, as required in particular by the observed $^3J_{C\text{-}1,C\text{-}3'}$.

The optical rotation expected for such conformational excursions would be very close to the observed value of $+120$ deg cm^2 dmol^{-1} (Fig. 1). There may be occasional excursions into the folded conformation of $\varphi,\psi = 180°, 0°$, which would be detectable neither by optical rotation nor by n.m.r.

The proposed conformational description avoids the entropically unlikely picture of extreme rigidity, yet allows for the substantial inflexibility of this disaccharide apparent at least in aqueous solution.

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