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Solution conformation of methyl β-xylobioside from optical rotation

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ABSTRACT

A recently developed semiempirical model of saccharide optical activity is used to calculate the Na_D rotation of methyl β-xylobioside in solution as a function of its conformation. Combining the results with published conformational-energy calculations leads to a picture in which folded conformations, with either ϕ or Ψ greater than 120°, make up no less than 40% of the total, in aqueous solution. This conclusion is consistent with an earlier analysis of the optical rotation of cellobiose in which folded forms were found to account for $< 10\%$ of the total.

INTRODUCTION

In order to describe the conformation of a carbohydrate in solution it is necessary to have a representation of its multidimensional potential-energy surface, and much effort is currently being directed at developing theoretical molecular-modeling methods for that purpose[1]. No single molecular-modeling algorithm or protocol has yet emerged through consensus, partly on account of the difficulty in verifying the accuracy of calculated energy surfaces through experiment. NMR methods serve as the major means of verification, but since the extent of molecular flexibility in solution is not generally known (and is, in fact, one of the objects of study), NMR data do not always have a unique interpretation.

Newly developed methods of interpreting optical-rotation data can complement NMR methods, such that their combined application can provide improved criteria for evaluating competing modeling approaches. Here we apply an optical-rotation based method of conformational analysis to methyl β-xylobioside (methyl 4-*O*-β-D-xylopyranosyl-β-D-xylopyranoside). Methyl β-xylobioside serves as a particularly simple and useful model compound by virtue of the absence of exocyclic hydroxymethyl groups.

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The linkage conformation of methyl β-xylobioside has been studied by X-ray diffraction[2–4], molecular modeling[5,6], and NMR methods[6,7]. The solid-state structure of xylobiose hexaacetate[4], in terms of the linkage dihedral angles ϕ(H-1–C-1–O-1–C-4′) and Ψ(C-1–O-1–C-4′–H-4′), is ϕ, Ψ, = 20°, −15°, similar to that of cellobiose. An early rigid-residue energy minimization[5] yielded a structure with ϕ, Ψ = 63°, 25°. More recently, a flexible-residue disaccharide calculation was carried out by Hricovini et al.[6], in which results using three current algorithms were compared. These included the semiempirical quantum-mechanical PCILO method (that is, perturbative configuration interaction using localized orbitals)[8] and two applications of the molecular-mechanics method, with an MM2 force field[9] and an MM2CARB force field[10]. Solvation energies were included with a continuum model[6]. Each of the calculated potential-energy surfaces displayed multiple low-energy conformations, with PCILO, MM2, and MM2CARB algorithms giving different distributions of these conformers in aqueous solution. The dominant conformation in water by PCILO was ϕ, Ψ = 62°, −152°; by MM2 ϕ, Ψ = 40°, −67°; and by MM2CARB ϕ, Ψ = 60°, 48°. Measured $^3J_{C,H}$ values could not be reproduced by averaging over the low energy conformers[6]. The conclusion must be reached that the solution conformation of methyl β-xylobioside has not yet been well established.

METHODS

The method we use to calculate the Na_D molar rotation, [M], has previously been described in detail[11], and its applicability to pyranoses[12], pyranosides[13], other model compounds[13,14], and disaccharides[15–17] demonstrated. Its main feature is the explicit reference to the high-energy optically active σ–σ^* electronic transitions deep in the vacuum UV which determine optical rotation and CD. It is based on the Kirkwood theory of optical activity[18,19] in which the high-energy electric-dipole-allowed transitions localized on an individual bond are summed to express the bond polarizability; the molecular optical rotation in the visible region of the spectrum is then described as the result of the interaction among bond polarizabilities. In our model the bond localized electric-dipole-allowed transitions are summed to express a single transition moment localized on each of the bonds. Perturbation theory is then used to describe their interaction, resulting in molecular transition moments, each of which is expressed as a linear combination of bond moments. The molecular transition moments, through well-known equations[20,21], determine the CD and, via a Kronig–Kramers transform, the optical rotation.

In the present work we used the same parameterization as was originally optimized for saccharide fragments (e.g., CH_2OH–CH_2OH)[11]. Each xylose residue in the chain was taken to be in the $^4C_1$(D) ring conformation with atomic coordinates adapted from the glucose coordinates of Arnott and Scott[22]. Hydroxyl hydrogen atoms do not enter into the calculation; their coordinates need not be specified.

Within the glycosidic linkage the C–O–C bond angle was taken[23–25] to be 117.5°. 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, $\Psi = 0°$ corresponds to the O-1–C-1 bond *cis* to the C′-4–H bond, and positive values of ϕ and Ψ refer to clockwise rotation of the reducing residue as viewed from the nonreducing residue. The linkage conformations we examined were those within the range of ϕ, Ψ values found by Hricovini et al.[6] to be free of extreme steric contacts.

The calculations refer to molecules in a vacuum. For comparison with aqueous solution data, a solvent correction $(n^2 + 2)/3 = 1.26$ can be applied, where n is the solvent refractive index[21]. We also apply an empirical scale factor of 1.69 to the calculated results, which brings monomer calculations into almost quantitative agreement with experimental data[15] and which may be related to optical activity contributions omitted in the Kirkwood theory. The uncertainty in the calculational model has been estimated[15] to be ± 24 deg cm^2 dmol^{-1}.

Methyl 4-*O*-β-D-xylopyranosyl-β-D-xylopyranoside was prepared by a condensation reaction in acetonitrile with mercuric cyanide as the catalyst, using 2,3-di-*O*-

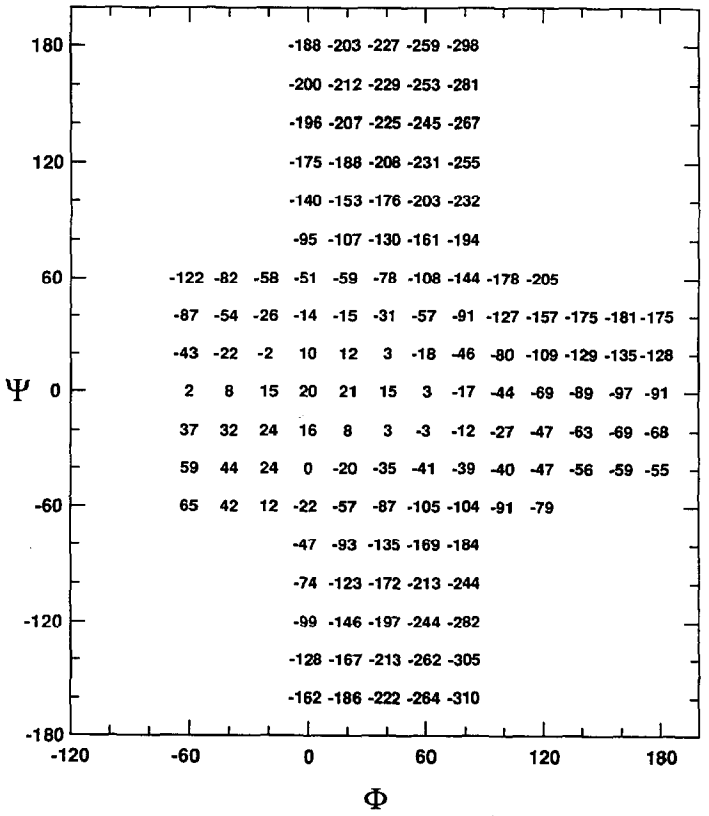

Fig. 1. Na_D molar rotations (in deg cm^2 dmol^{-1}) of methyl β-xylobioside calculated as a function of linkage dihedral angles ϕ and Ψ, with the methoxy group in the *gt* conformation.

acetyl-4-*O*-benzyl-α,β-D-xylopyranosyl bromide and methyl 2,3-di-*O*-acetyl-β-D-xylopyranoside as the glycosylating agent and nucleophile, respectively[26], Removal of protecting groups (catalytic hydrogenolysis and deacetylation) from isolated methyl *O*-(2,3-di-*O*-acetyl-4-*O*-benzyl-β-D-xylopyranosyl)-(1 → 4)-2,3-di-*O*-acetyl-β-D-xylopyranoside afforded the desired methyl β-(1 → 4)-xylobioside, mp 148–149°C.

Molar rotations, [M], were measured in water (-221 deg cm^2 dmol^{-1}), methanol (-167 deg cm^2 dmol^{-1}), dimethyl sulfoxide (-162 deg cm^2 dmol^{-1}), and 1,4-dioxane (-192 deg cm^2 dmol^{-1}), with a Perkin–Elmer Model 141 polarimeter.

RESULTS

Fig. 1 shows the calculated molar rotation, [M], of methyl β-xylobioside in aqueous solution as a function of linkage conformation, when the methoxy group is in the *gt* conformation. Fig. 2 shows the corresponding results with the methoxy group in the *tg* conformation.

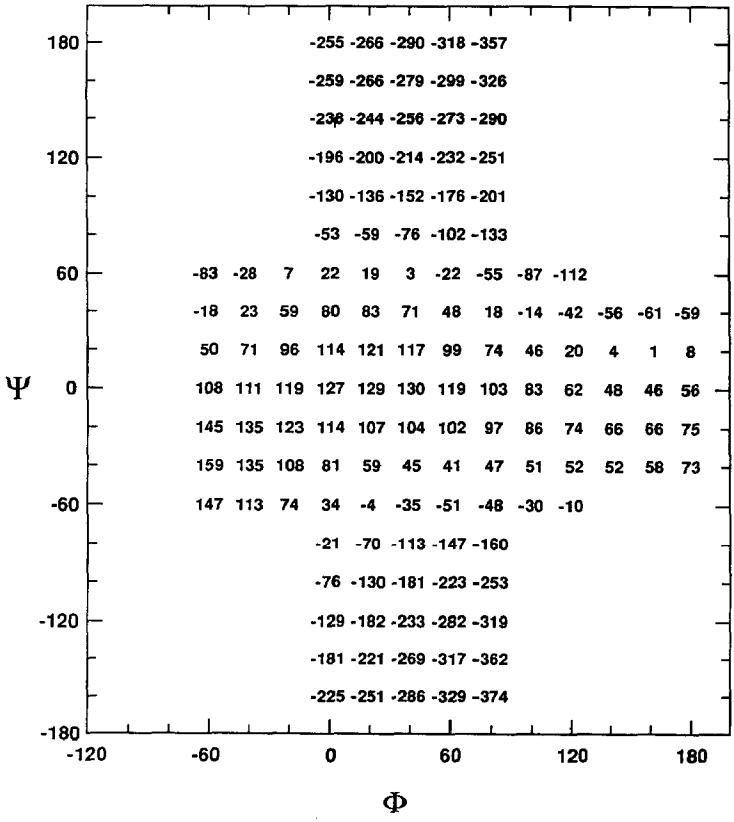

Fig. 2. Na_D molar rotations (in deg cm^2 dmol^{-1}) of methyl β-xylobioside calculated as a function of linkage dihedral angles ϕ and Ψ, with the methoxy group in the *tg* conformation.

TABLE I

Calculated low-energy conformations of methyl β-xylobioside with population distributions, statistically averaged calculated optical rotations, and observed rotations (in deg cm^2 $dmol^{-1}$).

Structure	(ϕ, Ψ)	Calculated population (%)							$[M]^{calc}$
		PCILO^{vac}	PCILO^{diox}	PCILO^{Me_2SO}	PCILO^{MeOH}	PCILO^{aq}	MM2^{aq}	MM2CARB^{aq}	
X1	39.6°, −67.4°	40.6	41.4	40.5	37.8	21.6	41	10	−105
X2	60.4°, 48.4°	41.1	37.8	29.7	24.5	10.3	26	48	−79
X3	62.4°, −152.4°	3.3	6.5	15.4	22.7	54.7	1	17	−270
X4	60.8°, −128.9°	0	0	0	0	0	0	0	−255
X5	−35.9°, −49.5°	3.4	2.6	1.6	1.4	0.3	29	3	39
X6	118.9°, −50.7°	7.8	6.2	4.5	3.6	1.2	0	0	−59
X7	153.5°, 29.9°	3.7	5.5	8.3	9.8	11.8	3	22	−156
	$\langle[M]\rangle^{calcd}$	−93	−108	−136	−137	−198	−60	−127	
	$[M]^{obsd}$		−192	−162	−167	−221	−221	−221	

Given the observed rotation of $[M] = -221$ deg cm^2 dmol^{-1} in aqueous solution, several conclusions follow directly from inspection of Figs. 1 and 2. First, the solid-state structure of ϕ, $\Psi = 20°$, $-15°$, observed in xylobiose hexaacetate[4], does not persist as the predominant structure in aqueous solution. The calculated rotation for that linkage geometry is 200–300 deg cm^2 dmol^{-1} more positive than the experimental value, which is well outside the uncertainty of the calculational model.

Also, conformations near ϕ, $\Psi = 60°$, 20° or ϕ, $\Psi = 60°$, 40° cannot represent the sole dominant species in aqueous solution. The calculated rotations for such conformations are 150–300 deg cm^2 dmol^{-1} more positive than the experimental rotation. This result is of interest because conformations in this region have been found to be predominant, by optical rotation analysis, in (1 → 4)-β-D-galactan[27] and (1 → 4)-β-D-mannan[28]. In these conformations O-1–C-4′ is *gauche* to C-1–O-5 and the conformations are stabilized by a favorable O-5–C-1–O-1–C-4′ torsional contribution, including any exoanomeric effect that may be present.

Calculated rotations approaching the magnitude of the observed rotation result only with conformations that can be characterized as being "folded" (i.e., having ϕ or $\Psi > 120°$). Thus, either methyl β-xylobioside has a predominant conformation that is folded, or there is substantial flexibility, resulting in a distribution of conformations in which these folded forms are significantly populated.

The conclusions drawn here are independent of the orientation of the methoxy group. The *gt* conformation is likely to be preferred over the *tg* conformation on account of the steric hindrance in the latter which results from the presence of the equatorial C-2′ hydroxyl group.

A more detailed application of the calculated results is possible by combining them with calculated conformational population distributions which result from one or another molecular modeling algorithm (see Discussion).

DISCUSSION

Hricovini et al.[6] found seven local energy minima in a search of ϕ, Ψ-space using PCILO methods; the energy minimized conformations are indicated in Table I together with relative populations in vacuum (PCILO^{vac}). They used a continuum model to evaluate solvent contributions to energy, and found that structure X3 was preferentially solvent stabilized to an extent proportional to the polarity of the solvent (PCILO^{diox}, PCILO^{Me_2SO}, PCILO^{MeOH}, and PCILO^{aq} populations in Table I). MM2 and MM2CARB methods of energy minimization did not affect the geometries of the low energy structures but did change the relative energies; Table I shows the calculated population distributions in water (MM2^{aq} and MM2CARB^{aq}).

The calculated optical rotations for the seven minimized structures in water are shown in the last column of Table I. For other solvents the values should be multiplied by the factor $[(n^{solv})^2 + 2]/[(n^{aq})^2 + 2]$, where n is the index of refrac-

tion; the relevant multiplicative factors are 1.06 for dioxane, 1.11 for Me_2SO and 1.00 for methanol.

Across the bottom of Table I are shown the statistically averaged optical rotations for each calculated population distribution, and the measured rotations.

Aqueous solution.—The PCILO^{aq} distribution of conformations gives a calculated averaged rotation similar to the observed rotation (Table I). Given the combined uncertainties of the optical rotation and solvation models, the difference between the observed rotation and that calculated with the MM2CARB^{aq} distribution may not be significant. The MM2^{aq} distribution gives poor agreement between calculated and observed rotation. It should be pointed out that the population distributions[6] and, therefore, the average optical rotation values are the result of averaging only over the set of local minimum energy conformations, not over the complete ensemble described by the entire energy surface. This approximation introduces an additional uncertainty.

The PCILO^{aq} and MM2CARB^{aq} distributions are not entirely dissimilar. In both, virtually all of the partition function comes from four conformations, X1, X2, X3 and X7. X1 and X2 are closely related extended conformations, in which O-1–C-4′ is *gauche* to C-1–O-5 ($\phi = 40$–$60°$), and in which the C-1–O-1–C-4′–H-4′ *cis* conformation ($\Psi = 0°$) is avoided with changes in Ψ either to approximately $+60°$ or $-60°$. The combined weight of these two conformations is substantial in both distributions (32–58%). X3 and X7 conformations are likewise related and represent extremely folded structures in which the mean planes of the two xylose rings are approximately perpendicular to one another, with either ϕ or Ψ approximately equal to 180°. The combined weight of X3 and X7 conformations is 67% in PCILO^{aq} and 39% in MM2CARB^{aq}. The main difference between the PCILO^{aq} and MM2CARB^{aq} distributions is that the extended conformations (X1 + X2) predominate in MM2CARB^{aq} whereas the folded conformations (X3 + X7) predominate in PCILO^{aq}. The fact that the folded conformations are expected to display optical rotation of larger magnitudes than the extended conformations (Table I) leads to the difference in averaged rotation derived from the two distributions.

A major conclusion of the present work is that folded conformations make up no less than 40% of the conformers of methyl β-xylobioside in aqueous solution. A large population of folded forms is required to account for the optical rotation, and the stability of folded forms is apparent in the energy calculations. The stabilization of folded forms described by the PCILO^{aq} distribution (67%) seems extreme and the apparent closer agreement of observed and calculated rotations for that distribution should not be taken as definitive because of the various uncertainties involved (above).

The present result can be compared with the corresponding result obtained in an earlier optical rotation analysis of cellobiose[15], in which it was concluded that similarly folded conformations account for $< 10\%$ of the conformers in aqueous solution. In cellobiose unfavorable steric interactions between the 6-hydroxymethyl

group on the reducing residue and the ring oxygen atom of the nonreducing residue provides a reasonable explanation for the larger population of folded conformations in xylobioside.

A more detailed conformational analysis is probably not justified at the present time, which leaves open the questions of which extended form (X1 or X2) and which folded form (X3 or X7) predominate in aqueous solution.

Nonaqueous solutions.—The observed solvent dependence of optical rotation is much less than the variation in optical rotation calculated from the PCILO distributions (Table I). The electrostatic contribution to calculated solvation energy seems to be a major determinant of the large solvent dependence of calculated distributions[6]. An overevaluation of that contribution would lead both to an overestimation of the fraction of folded forms and an overestimation of the solvent dependence of optical rotation. Thus, if in going from vacuum to water, the percentage of X3 conformations increases to a value less than the $PCILO^{aq}$ value of 55%, the variation in calculated average rotation would be less than the two-fold change displayed in Table I.

In any case, the observed solvent dependence of optical rotation is too large to be accounted for by a simple consideration of variation in index of refraction and presumably reflects a difference in conformation distributions. Some dependence of the conformation distribution on solvent is also indicated in the variation of $^3J_{C,H}$ values previously reported[6]. That the dependence is relatively small is supported by the similarity of measured NOEs in water and methanol[7].

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