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NUCLEAR OVERHAUSER EFFECT AND CONFORMATIONAL STATES OF CELLOBIOSE IN AQUEOUS SOLUTION

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ABSTRACT

A nuclear Overhauser enhancement in α-cellobiose 1-phosphate, resulting from pre-irradiation of H-1′ of the non-reducing glucosyl group, was measured and calculated theoretically. Comparison of these data reveals a complicated conformational equilibrium in aqueous solutions of the cellobiose derivative.

INTRODUCTION

A knowledge of the conformational states of disaccharide units is essential in considering the spatial arrangement of polysaccharide chains, especially in solution. In this context, we now report experimental data and theoretical calculations for cellobiose derivatives in aqueous solution.

Theoretical calculations on the conformations of cellobiose have been carried out[1–5]. The results showed that the use of various versions of the semiempirical hard-sphere calculations, with different parameters for the force field, leads to different quantitative and even qualitative conclusions. Thus, the calculations by Melberg and Rasmussen[4] predicted a statistical weight of merely 1% for the structure with the C-1′–H-1′ bond *trans* to the C-4–H-4 bond (Fig. 1). For such a structure, one of the rotation angles around the glycosidic linkage ψ is ~180°. We have shown[5] that the statistical weight of such conformations may be ~13%.

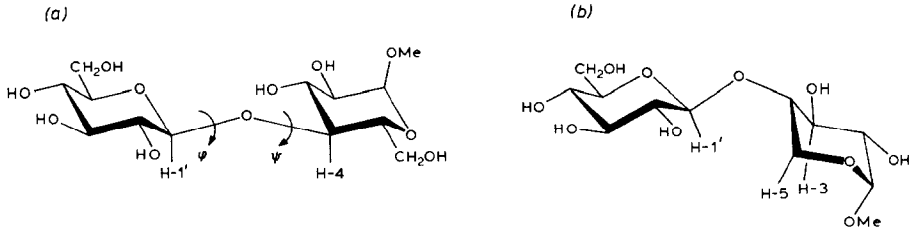

Fig. 1. Molecular models of methyl α-cellobioside conformations with rotation angles φ (C-1′–O) and ψ (O–C-4) 60 and 10° (*a*), 30 and 180° (*b*); φ is zero for the *cis* orientation of C-1′–H-1′ and O–C-4′ in the H-1′–C-1′–O–C-4 fragment; ψ is zero for the *cis* orientation of C-1′–O and C-4–H-4 in the C-1′–O–C-4–H-4 fragment.

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Until recently, the conformational states of cellobiose in aqueous solution were deduced from $^3J_{C,H}$ values for the carbons and protons near the glycosidic linkage[6], and from the linkage rotation[7] Λ. The n.O.e. resulting from pre-irradiation of H-1′ provides direct information on the conformational equilibrium of cellobiose in aqueous solution. α-Cellobiose 1-phosphate was chosen for n.O.e. measurements since the different configurations at the two glycosidic centres provide sufficient resolution of the ^{1}H-n.m.r. spectrum. The configuration of the centres remote from the glycosidic linkage cannot significantly affect its conformational properties; the $^3J_{C,H}$ values for α-cellobiose 1-phosphate are almost identical to those for the methyl β-glycosides of cellobiose[6] and lactose[8].

A theoretical conformational analysis was carried out for methyl α-cellobioside. Since the observed values are statistically averaged, we have calculated the average values of n.O.e., $^3J_{C,H}$, and Λ, taking into account the whole range of possible conformations. Comparison of the observed and calculated data allows the conformational flexibility of cellobiose to be assessed more reasonably.

In evaluating the potential energy of conformers, account was taken of non-bonded interactions, estimated using the functions of Scott and Scheraga[9]. The torsional barrier for rotation around the glycosidic linkages was assigned a value[10] of 0.9 kcal/mol. The electrostatic interactions, the formation of intramolecular hydrogen-bonds, and the *exo*-anomeric effect were negelected since, in aqueous solution, these factors play an insignificant role[5].

The spatial structure for the reducing glucose residue of the disaccharide was taken from neutron diffraction data[11] for methyl α-D-glucopyranoside and that for the non-reducing glucosyl group from the X-ray analysis[12] of methyl β-D-glucopyranoside. An average value of 1.1 Å was taken for the length of the aliphatic C–H bonds, and the glycosidic bond angle was assigned a value[13] of 116.7°.

The n.O.e. observed for the proton d when the proton s is saturated is given by the following expression[14], where r_{ds} is the distance between the protons d and s:

$$f_s^d = r_{ds}^{-6}/2 \sum_{k \neq d} r_{dk}^{-6} - \sum_{k \neq d,s} (r_{dk}^{-6} \cdot f_s^k)/2 \sum_{k \neq d} r_{dk}^{-6}.$$

The refined values of f_s^d were taken by iterations. For the conformation of the disaccharide, defined by the rotation angles φ and ψ, the corresponding value of $(f_s^d)^{\varphi,\psi}$ and its Boltzmann probability were calculated. This allows the average value of the n.O.e. $\langle f_s^d \rangle$ to be estimated.

The $^3J_{C,H}$ values (J^φ and J^ψ) for the H-1′–C-1′–O–C-4 and C-1′–O–C-4–H-4 fragments of cellobiose, defined by the rotation angles φ and ψ, can be determined for some conformations *via* their experimental dependence on the rotation angles obtained by Perlin and co-workers[6]. The relation between the linkage rotation Λ and the angles φ and ψ was reported by Rees[15].

RESULTS AND DISCUSSION

The ^{1}H-n.m.r. spectrum of α-cellobiose 1-phosphate is shown in Fig. 2a. The principal signals were assigned using the method of selective homonuclear resonance in both the usual and difference versions. Fig. 2b shows the difference n.O.e. spectrum obtained by pre-iradiation of H-1′, in which the signals of H-4, H-5, and an unresolved multiplet (between the resonances of H-3 and one H-6) were enhanced. Since an upfield component of the triplet from H-3′ (designated in Fig. 2 by an arrow) was clearly observed, the occurrence of a positive n.O.e. for this proton is beyond doubt. For H-2′, which interacts with H-1′ *via* a spin–spin mechanism, a typical "pseudo-INDOR" picture was observed. For H-5′ and H-3′, an overall n.O.e. was measured since these signals overlap. The n.O.e. for H-4, H-5, and the overall values for H-6 and H-3, as well as for H-5′ and H-3′, are shown in Table I.

Four local minima corresponding to the optimum conformers A–D were found on the conformational map for methyl α-cellobioside calculated by taking into account the non-bonded interactions (*cf.* Fig. 2 in ref. 5). The values of φ and ψ and the energies of these conformers are shown in Table II. The structure D is of particular interest since ψ has a value of $\sim 180°$ that was not found by X-ray analysis in di- and oligo-saccharides. Despite the differences in φ and ψ in the structures A–C, H-1′ and H-4 are essentially in close proximity (2.3 Å). In contrast, for D,

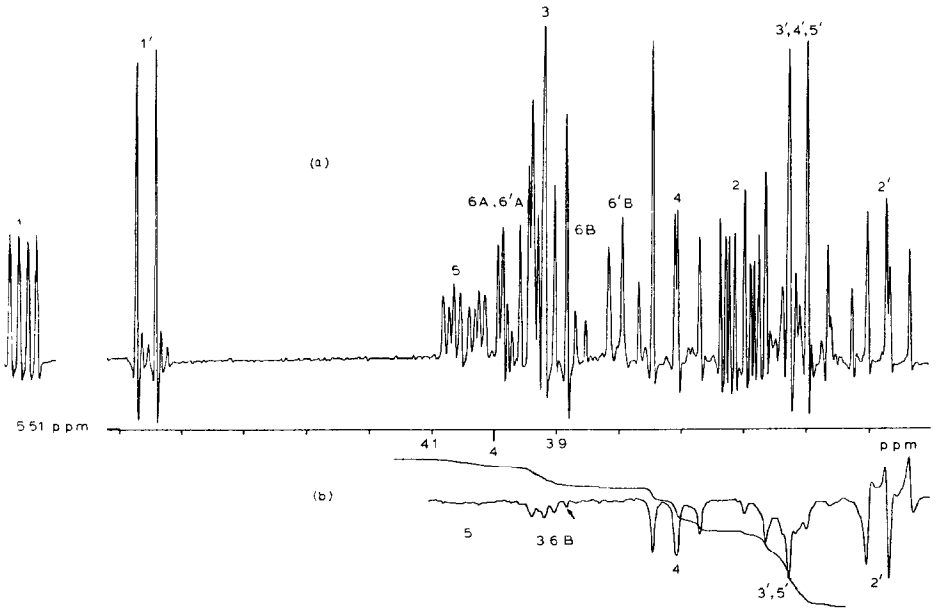

Fig. 2. (*a*) ^{1}H-N.m.r. spectrum of α-cellobiose 1-phosphate (primed numbes refer to the protons of the non-reducing unit), (*b*) fraction of the difference n.O.e. spectrum.

TABLE I

OBSERVED n.O.e.'s ON PRE-SATURATION OF H-1′ OF α-CELLOBIOSE 1-PHOSPHATE

Proton(s)	*N.O.e. (%)*
H-3′ + H-5′	15
H-5	2
H-3 + H-6	4.2
H-4	8.8

these atoms are reasonably remote (3.5 Å), and H-1′ is located ~2 Å from both H-3 and H-5 (Fig. 1).

The statistical sums of the domains corresponding to the spatial forms A–D can be inferred from the potential surface, and the overall equilibrium can be estimated. The latter is characterised by the contribution of the forms A–D of 40, 44, 6, and 10%, respectively (Table II). This distribution implies that the contribution of a conformer is essentially defined by the entropy factor, since enthalpies of all four optimum structures of methyl α-cellobioside are practically equal (Table II).

N.O.e.'s were calculated for H-3, H-4, H-5, and H-6 (Table II) since only for these protons were they established experimentally (Table I). The hydroxymethyl groups of the glucose residues were fixed in two possible orientations[16] (gt and gg). The n.O.e.'s for the four optimum conformations of methyl α-cellobioside are given in Table II, and the average values in Table III. Since n.O.e. measurements are dependent on the experimental conditions[14], subsequent analysis will be carried out in terms of relative n.O.e.'s.

TABLE II

TORSIONAL ANGLES (°), ENERGIES (kcal/mol), n.O.e.'s (%), COUPLING CONSTANTS (Hz), AND LINKAGE ROTATION (°) FOR THE OPTIMUM CONFORMATIONS OF METHYL α-CELLOBIOSIDE

Parameters	*Conformers*			
	A	*B*	*C*	*D*
φ, ψ	30, 40	60, 10	−20, −20	30, 180
U	−3.1	−3.1	−2.8	−2.7
$f^{H\text{-}4}_{H\text{-}1'}$	19	15	16	−1
$f^{H\text{-}3}_{H\text{-}1'}$	1	−1	0	32
$f^{H\text{-}5}_{H\text{-}1'}$	0	−1	0	27
$f^{H\text{-}6A}_{H\text{-}1'}$ [a]	0	7	0	−1
J^{φ}	4.3	2.0	4.8	4.3
J^{ψ}	3.5	5.1	4.8	6.1
Λ	+122	−25	+187	+45
Statistical weights (%)[b]	40	44	6	10

[a]H-6A is *trans* to the C-5–O-5 and *gauche* to the C-4–C-5 bond. [b]Calculated from the free energies of the conformers.

TABLE III

AVERAGE VALUES OF n.O.e.'s, J^{φ} AND J^{ψ} (Hz), Λ (°), AND ROTATION ANGLES φ,ψ FOR METHYL α-CELLOBIOSIDE

Parameters	*Present calculation*	*Calculation[a] by the HSEA method*
$\langle\varphi\rangle, \langle\psi\rangle$	34, −20	60, 0
$\langle f_{H\text{-}1'}^{H\text{-}4}\rangle$	16.6	17.4
$\langle f_{H\text{-}1'}^{H\text{-}3}\rangle$	2.9	−1
$\langle f_{H\text{-}1'}^{H\text{-}5}\rangle$	1.5	−1
$\langle f_{H\text{-}1'}^{H\text{-}6A}\rangle$	2.5	2.8
$\langle J^{\varphi}\rangle$	3.7	2
$\langle J^{\psi}\rangle$	4.8	5.3
$\langle\Lambda\rangle$	+65	0

[a]Parameters are determined for the conformer with φ 60° and ψ 0°.

An immediate consequence of the positive n.O.e.'s observed for H-3, H-5, and H-6 is the occurrence of the conformers B and D in aqueous solution of α-cellobiose 1-phosphate, since the positive n.O.e. on H-6,6 can be in conformer B only, whereas D yields positive n.O.e.'s on H-3 and H-5 (Table II). The contribution of D to the overall equilibrium is small; this follows from the fact that, for D, the calculated values $f_{H\text{-}1'}^{H\text{-}3}$ and $f_{H\text{-}1'}^{H\text{-}5}$ amount to 32 and 27%, but $f_{H\text{-}1'}^{H\text{-}4}$ is close to zero, whereas the observed n.O.e. for H-4 is several times greater than that for H-5. Actually, the average values $\langle f_{H\text{-}1'}^{H\text{-}5}\rangle$ and $\langle f_{H\text{-}1'}^{H\text{-}3}\rangle$ are rather small (1.5 and 2.9%, respectively, Table III).

The actual content of form D seems to exceed the calculated value (10%, Table II). This follows from the correlation between the theoretical (1:10, Table III) and observed (1:5, Table I) ratio of the average values $f_{H\text{-}1'}^{H\text{-}5}/f_{H\text{-}1'}^{H\text{-}4}$. Form D demonstrates a wide range of ψ.

In deducing the content of the spatial form B from the n.O.e.'s on H-6,6, it is noteworthy that only the sum of n.O.e.'s on H-3 and H-6 (4.2%, Table I) can be determined. Nevertheles, since the values $f_{H\text{-}1'}^{H\text{-}3}$ and $f_{H\text{-}1'}^{H\text{-}5}$ for D are close (Table II), it is reasonable to assume that the n.O.e. for H-3 is approximately the same as for H-5, *i.e.*, 2% (Table I). Therefore, the n.O.e. for H-6A should be 2%. Thus, the observed ratio $f_{H\text{-}1'}^{H\text{-}6}/f_{H\text{-}1'}^{H\text{-}4}$ is ~1:5, whereas that calculated for the optimum conformation B is 1:2 (Table II). Hence, form B, together with other conformers, occurs in aqueous solutions of α-cellobiose 1-phosphate. The calculated contribution of B is 44% (Table II). The observed ratio $(f_{H\text{-}1'}^{H\text{-}6} + f_{H\text{-}1'}^{H\text{-}3})/f_{H\text{-}1'}^{H\text{-}4}$ is 1:2 (Table I). The ratio of the calculated average values of the above n.O.e.'s is 1:3 (Table III). Thus, the agreement with the experimental data is satisfactory.

The fact that conformer B fails to be a unique spatial form of cellobiose in aqueous solution follows unambiguously from the data on linkage rotation. Only this structure yields a negative linkage rotation (Λ −25°, Table II), whereas the observed value is positive[7] (Λ +59°). In an aqueous solution of cellobiose, together

with B, there must occur another conformer of considerable statistical weight, with a positive linkage rotation and lacking n.O.e.'s on H-6,6. The spatial form A (Λ +122°) evidently meets the above requirements (Table II). The observed value (+59°) of Λ is practically the mean of the linkage rotations of conformers A and B. This fact clearly indicates an approximately equal contribution of A and B to the conformational equilibrium of cellobiose. The calculated statistical weights of A and B are in accordance with this suggestion (Table II).

The contribution of the fourth structure C appears to be negligible (~6%, Table II). The low probability of conformer C having a *trans*-orientation of the aglycon with respect to the C-1′–O-5′ bond follows from the calculation that takes into account the free energies of the conformers, without any additional restrictions associated with the interaction of lone pairs of O-5′ and O-1′.

Thus, the conformational distribution for cellobiose in aqueous solution now proposed (40, 44, 6, and 10%, respectively, for A–D) may be regarded as reasonable. Such a distribution allows the observed n.O.e.'s, linkage rotation Λ, and J^{φ} and J^{ψ} values to be accounted for. Thus, the calculated $\langle\Lambda\rangle$ = +65° (Table III) accords with that[7] (+59°) observed. Further, the average values $\langle J^{\varphi}\rangle$ 3.7 and $\langle J^{\psi}\rangle$ 4.8 Hz (Table III) proved to be close to those found[6] for methyl β-cellobioside (J^{φ} 4.2, J^{ψ} 4.3 Hz), and nearly coincident with those found[8] for methyl β-lactoside (J^{φ} 3.8, J^{ψ} 4.9 Hz). The data for methyl β-lactoside are more accurate since they were measured using a 600-MHz instrument. Hence, it follows that accounting for nonbonded interactions is sufficient to describe the conformational equilibrium in aqueous solutions of cellobiose.

Conformational analysis of methyl α-cellobioside using the HSEA approach[17] takes into account the vacuum function of the *exo*-anomeric effect. Only one conformer of methyl α-cellobioside (φ 60°, ψ 0°, *i.e.*, form B) is allowed. The coupling constants (J^{φ} 2, J^{ψ} 5.3 Hz, Table III) and linkage rotation (Λ 0°) are strikingly different from the values observed for cellobiose and its methyl glycosides (J^{φ} 4.2 Hz, Λ +59°). Restriction to conformer B fails to account for some fine effects revealed in the measurements of n.O.e.'s. For the rotation angles φ 60° and ψ 0°, the calculation gives small negative n.O.e.'s for H-3 and H-5 (−1%, Table II), whereas the observed values are positive (Table I). Thus, the structure B that is optimal with respect to the *exo*-anomeric effect is one of the spatial forms present in aqueous solutions of the disaccharide (Table I).

Great care must be taken in assigning a definite conformation to disaccharides. As an example, we point out that, on the basis of J^{φ} and J^{ψ} (~4.2 Hz), the absolute values of φ and ψ were deduced[6] to be 30°. Our calculation of the average values $\langle\varphi\rangle$ and $\langle\psi\rangle$, with allowance for the whole conformational distribution of cellobiose ($\Delta\varphi$ 100°, $\Delta\psi$ 180°), yields values of +35° and −20° (Table III). At the same time, such a "statistically averaged" conformation fails to reproduce Λ correctly and the n.O.e.'s on H-3, H-5, and H-6A. Thus, the experimental data and theoretical calculations indicate that cellobiose in aqueous solution is a conformationally labile disaccharide, and the range of the rotation angles φ (C-1′–O) and

ψ (O–C-4) is determined by the whole molecular potential surface of non-bonded interactions.

EXPERIMENTAL

^{1}H-N.m.r. spectra were recorded with a Bruker WM-250 instrument for 3% solutions of α-cellobiose 1-phosphate in D_2O at 30° (internal sodium 4,4-dimethyl-4-silapentanesulfonate). N.O.e.'s were measured on pre-irradiation of H-1′ and within the t.O.e. technique[18]. The relaxation delay D1 was 0.5 s and the build-up n.O.e. D2 was 0.8 s. N.O.e.'s were expressed as a ratio of the integrated intensities of the observed and presaturated proton.

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