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Structure and solution conformations of a cyclic trisaccharide from high-resolution n.m.r. spectroscopy and molecular modelling

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ABSTRACT

The conformational properties of a cyclic trisaccharide: [O-β-D-glucopyranosyl-(1→6)]$_3$ 1,6″-anhydride nonaacetate ($C_{36}H_{48}O_{24}$, **1**) have been established by high-resolution ^{1}H- and ^{13}C-n.m.r. spectroscopy in conjunction with potential-energy and molecular-mechanics calculations. The n.m.r. parameters used were nuclear Overhauser enhancements (n.O.e.) and coupling constants. From theoretical models of the trisaccharide, a statistical-mechanics approach was used to compute an ensemble average-relaxation matrix from which the n.O.e. were calculated. The observed nuclear Overhauser enhancements as measured by n.m.r. spectroscopy may be satisfactorily modelled if averaging over two conformational states is considered. In solution, both conformations of the molecule exhibit three-fold symmetry; the β-linked glucopyranose rings have the $^4C_1$ conformation. In one conformer, the orientation about the (1→6) linkage is characterized by torsion angles $\Phi = -79.5°$, $\psi = 143.5$, and $\omega = -64.3$. For the other conformer, these values are $\Phi = -137.7$, $\Psi = 68.2$, and $\omega = 45.6$. The existence of such a conformer shows that solution behaviour is not dominated by the stabilizing influence of the exoanomeric effect.

INTRODUCTION

The major spectroscopic tools for determining three-dimensional structures of molecules and macromolecules are X-ray diffraction and nuclear magnetic resonance (n.m.r.) spectroscopy. In the past decade, there has been an upsurge in the application of n.m.r. to the assessment of molecular conformations in solution. N.m.r. effects are of two types: those transmitted through bonds (such scalar effects as coupling constants) and those transmitted through space (dipolar effects, such as the nuclear Overhauser enhancement, n.O.e.). Provided that the distance dependence of the effect is well understood, as in the case of n.O.e.[1], through-space effects may be used to estimate internuclear distances. Applications to structure determinations include such complex natural products as proteins. Almost all applications of n.O.e. to conformational analysis assume, explicitly or implicitly, monoconformational behavior.

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The use of n.O.e. values in modelling of oligosaccharides requires two special precautions. First, as carbohydrate molecules contain many protons in close proximity, a simple two-spin treatment is not satisfactory, particularly in the analysis of steady-state enhancements. The full-relaxation matrix method is required, and a collection of simultaneous equations, relating all the n.O.e. values and all proton–proton distances, must be solved. The second difficulty arises because of the flexibility of carbohydrates at the glycosidic linkage. These molecules have extensive conformational space available and may sample this entire space during the time period needed for the build-up of n.O.e. Therefore, the experimental values reflect an average over all conformational states, corresponding to what has been termed the "virtual conformation" by Jardetzky[2]. A "virtual conformation" may have no physical significance; it may not correspond to any of the actual conformations adopted by the molecule and that can be assigned a finite lifetime. To deduce three-dimensional structures and conformations from n.O.e. measurements, one must be able to model such internal flexibility. Recently, ensemble average n.O.e. have been calculated for flexible disaccharides[3,4]. These studies used methods of statistical mechanics based on potential-energy surfaces calculated from the rotation of hexopyranoses about the glycosidic torsion-angles. When these ensemble average n.O.e. are compared to observed values, they are usually found in satisfactory agreement. However, different n.O.e. are predicted if different potential-energy surfaces are used[5,6].

Clearly, the determination of three-dimensional structures and conformations of carbohydrate molecules from n.m.r. measurements in solution is a rather complex process. The values of the experimentally observed n.m.r. data (n.O.e. and T_1) depend in a complex fashion on intimate molecular features, and have to be viewed as representative of the distribution of populations of low-energy conformers. There is thus a need to determine how structural features can be derived reliably from solution measurements. To this end, we have assessed the solution conformations of a molecule that exhibits limited internal flexibility. The present work is concerned with the investigation of the cyclic trisaccharide, [*O*-β-D-glucopyranosyl-(1→6)]$_3$ 1,6″-anhydride nonaacetate (**1**).

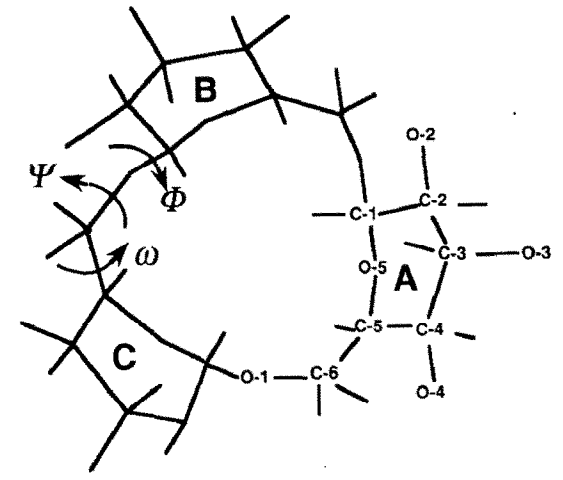
MATERIAL AND METHODS

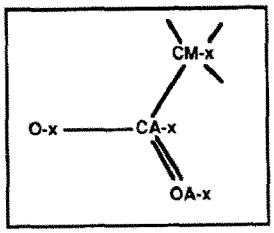
Sample. – The cyclic trisaccharide: [*O*-β-D-glucopyranosyl-(1→6)]$_3$ 1,6″-anhydride nonaacetate, (**1**) was prepared by internal cyclization of a bifunctionalized linear trisaccharide; details of the chemical synthesis of the peracetylated trisaccharide have already been published[7–10].

Nomenclature. – A schematic drawing of the cyclic trisaccharide is shown in Fig. 1, along with some torsional angles of interest. Indices A, B, and C are assigned, in an arbitrary fashion to the glucose residues.

Glycosidic junction through a (1→6) linkage between two residues j and $j+1$ offers three contiguous variable torsion angles: Φ, Ψ, and ω where:

$\Phi = O_j\text{-}5\text{-}C_j\text{-}1\text{-}O_j\text{-}1\text{-}C_{j+1}\text{-}6$





6)-β-D-Glcp-(1→6)-β-D-Glcp-(1→6)-β-D-Glcp-(1→

1

Fig. 1. Schematic drawing of the molecule of [*O*-β-D-glucopyranosyl-(1→6)]$_3$ 1,6″-anhydride nonaacetate **1**, with labelling of the atoms and torsion angles of interest. The atoms of the acetate groups are labeled CA, CM, and OA (inset). These abbreviations denote carbonyl C, methyl C, and carbonyl O, respectively.

$\Psi = C_j\text{-}1\text{–}C_j\text{-}1\text{–}C_{j+1}\text{-}6\text{–}C_{j+1}\text{-}5$

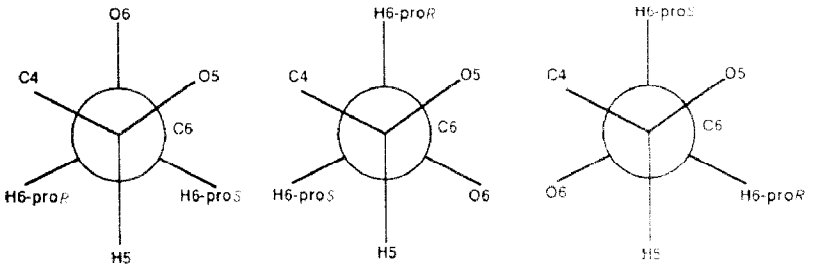
$\omega = O_j\text{-}1\text{–}C_{j+1}\text{-}6\text{–}C_{j+1}\text{-}5\text{–}O_{j+1}\text{-}5.$

The orientation about ω is referred to as either *gauche-trans* (*gt*), *gauche-gauche* (*gg*), or *trans-gauche* (*tg*). In this terminology, the torsion angle: O_j-1–C_{j+1}-6–C_{j+1}-5–O_{j+1}-5 is stated first, and the torsion angle: O_j-1–C_{j+1}-6–C_{j+1}-5–C_{j+1}-4, second.

The two prochiral protons at C-6, H-6pro*R* and H-6pro*S*, may be differentiated by the rule proposed by Hanson[11], by selective deuteration, and by n.m.r. results on model compounds[12,13]. Their relative orientations as a function of the rotation about C-5–C-6 are depicted in Fig. 2.

N.m.r. spectroscopy. — ^{1}H- and ^{13}C-n.m.r. spectra were recorded at 303 K with Bruker AM-300 or/and AM-400 spectrometers equipped with Aspect 3000 computers. The sample of **1** was dissolved in $CDCl_3$ (30 mg per mL). Chemical shifts are expressed in p.p.m. relative to Me_4Si as internal standard.

The inversion–recovery method was used for T_1 determinations [two pulse-sequences (T...180...*t*...90)n] with at least 12 *t* values. The peak heights of the different signals were measured as a function of delay time (*t*) and these data were analyzed by computer to determine the relaxation rates ($\sim$10%).



	gg	*gt*	*tg*
O-5—C-5—C-6—O-6 :	-60°	+60°	180
C-4—C-5—C-6—O-6 :	+60°	180°	-60
H-5—C-5—C-6—H-6pro *R* :	+60°	180°	-60
H-5—C-5—C-6—H-6pro *S* :	-60°	+60°	180

Fig. 2. Newman projections showing the identification of hydrogen atoms H-6pro*R* and H-6pro*S*, for the three non-eclipsed conformations *gauche-gauche* (*gg*), *gauche-trans* (*gt*), and *trans-gauche* (*tg*) about C-5 C-6.

^{13}C ^{1}H Nuclear Overhauser enhancements were derived from the ratio of the intensity of fully decloupled spectra to the intensity of spectra in which the proton-noise decoupler was gated off during at least 8 T_1 values, to eliminate the n.O.e.[14]. The estimated error was 15%.

Proton n.O.e. determinations were performed on degassed $CDCl_3$ solutions according to the method of Kinns and Sanders[15], which consists of saturating individually and selectively each line of the proton multiplet resonances but with low power decoupling, thus giving more reliable results.

Potential-energy calculations. — In a first step, the conformational energy is evaluated by including the partitioned contributions arising from the van der Waals interactions, torsional factors, and the exoanomeric effect. This method, referred to as PFOS (Potential Functions Oligosaccharide Structures)[16], has been shown to yield a satisfactory qualitative description of the conformational-energy surface for oligosaccharides[17]. The non-bonded interactions are evaluated by using 6–12 potential functions with the parameters proposed by Scott and Scheraga[18]. A three-fold sinusoidal potential is used for rotation about the glycosidic torsion angle Ψ, with a barrier of 1.0 kcal/mol for the eclipsed conformations. For rotations about the glycosidic C-1–O-1 linkage, namely the Φ angles, the intramolecular mechanism responsible for the exoanomeric effect is taken into account using the potential functions proposed by Tvaroska[19]; a sinusoidal three-fold eclipsed rotational barrier of 1.0 kcal/mol is also included. As a peracetylated trisaccharide molecule is being modelled, there is no need to take into account intramolecular hydrogen-bonding. Similarly, no electrostatic interaction is considered. For each disaccharide unit, and for a fixed orientation of the torsional angle ω, a potential-energy surface is calculated as a function of the glycosidic torsional angles Φ and Ψ at a given interval. Iso-energy contours are drawn by interpolation of 1 kcal/mol with respect to the energy minimum for the disaccharide unit

in question. This interpretation implies that, in a first step, all contributions to the total energy of the molecule arise from nearest-neighbour residue–residue interactions.

In a second step, the low-energy conformers are submitted to a complete energy optimization through molecular-mechanics calculations[20] using the MM2P-85 version (Molecular Mechanics Calculations[21]). Contributions arising from stretching, bending, stretch-bending, torsional, dipolar, and non-bonded energies are taken into account. In such a type of calculation, all of the atoms, including the hydrogen atoms, as well as the lone pairs on the oxygen atoms, have to be considered.

Modelling strategy. — Modelling studies were conducted on the trisaccharide and its peracetylated derivative. The starting geometry for the glucose residues was taken from the standard residues derived from the Carbohydrate Standard Fragment Library[22]. The coordinates of the hydrogen atoms were determined by using a C–H bond length of 0.11 nm and a bond vector related appropriately to the C–C and C–O bond vectors. As for the peracetylated trisaccharide, the sugar ring geometry (a slightly distorted $^4C_1$ conformation) was selected from a survey of acetylated glucose residues[23]. The coordinates of the hydrogen atoms were determined as already described. The atoms of the acetate groups are labelled CA, CM, and OA, denoting the carbonyl C, the methyl C, and the carbonyl O atoms respectively. As in other carbohydrate acetates, the secondary acetate groups are arranged in such a way that the carbonyl group eclipses the axial hydrogen atom at the corresponding ring-carbon atom[24].

It has already been pointed out[25] that the construction of a cyclic oligosaccharide may be performed with the use of helical parameters, n and h. In this instance, n is the number of residues, and h the advance per residue, which is the translation along the helix axis, and has to be set equal to zero. In the present work, calculation of the helical parameters as a function of variations of Φ and Ψ (ω being assigned a fixed value at given intervals) was performed following an algorithmn reported previously[25].

Calculation of n.O.e.. — The method for calculating theoretical n.O.e. values from an ensemble of possible conformations has been described by Cumming and Carver[3], and is described only briefly here. For all possible conformations, their probability of existence (P_i) with respect to relative polulation depends upon their energies and follows a Boltzmann distribution with a partition function Q. For a system having i different microstates we have:

$$P_i = \exp(-E_i/kT)/Q \qquad (1)$$

and

$$Q = \Sigma \exp(-E_i/kT) \qquad (2)$$

The averaged $<f(d,s)>$ (n.O.e. value detected on proton d when irradiating proton s) may be calculated by solving a series of simultaneous equations[1,26] from the matrix of all averaged $<r(k,l)^{-6}>$. In each of the i microstates, the conformation is fixed and $r(k,l)$ is the distance between proton k and proton l. Each term of the averaged distance-matrix may then be calculated: $<r(k,l)^{-6}> = \Sigma P_i r_i(k,l)^{-6}$. (3)

RESULTS AND DISCUSSION

Symmetry of the molecule and conformation of the pyranose rings. — The ^1H and ^{13}C spectra of **1** show that the three glucopyranosyl residues are magnetically equivalent about a C_3 symmetry axis. The ^1H and ^{13}C signals were assigned by 2D COSY and 2D heteronuclear correlated ^1H and ^{13}C experiments, respectively[27]. ^1H chemical shifts and coupling constants, along with ^{13}C chemical shifts, are presented in Tables I and II, respectively. The magnitude of the following coupling-constants $J_{1,2}$ (7 Hz), and $J_{2,3}$, $J_{3,4}$, $J_{4,5}$ ($\sim$ 9.5 Hz), are characteristic of a glucopyranose ring having the $^4C_1$ chair conformation. Furthermore, the magnitude of the coupling constants $J_{1,2}$ (7 Hz) and $^1J_{C-1,H-1}$ (162 Hz) confirm that the three glucopyranosyl residues are β linked.

Identification of the prochiral protons at C-6. — The prochiral methylenic hydrogen atoms at C-6 may be differentiated by the rule proposed by Hanson[11]. Their identification is always problematic: one way to make unambiguous assignment would require selective deuteriation of one of the two positions. These two hydrogen atoms have been unambiguously identified in the case of glucose, and of its derivatives, by chemical deuteration[12,13]. Two distinct ^3J coupling constants between H-5 and and H-6s were measured. The smaller corresponds to the coupling between H-5 and H-6pro*S*; conversely the larger corresponds to the coupling between H-5 and H-6pro*R*. Therefore, the signal at low field (3.67 p.p.m., $J_{5,6}$ 1.5 Hz) was assigned to H-6pro*S*, whereas the signal at high field (4.07 p.p.m., $J_{5,6}$ 6.4 Hz) was assigned to H-6pro*R*. These assignments are in agreement with the H-6pro*R*, H-6pro*S* chemical shifts observed in glucopyranose monomers. However, different assignments are proposed for the ^1H-n.m.r. data of gentiobiose peracetate[28] and gentiobiose in D_2O, where[29] the resonance of H-6pro*R* is at a lower field than that of H-6pro*S*.

TABLE I

^1H Chemical shifts (δ) and coupling constants (Hz) for **1**[a]

	H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6proS*	*H-6pro*R
δ	4.65	4.95	5.25	5.03	3.79	3.67	4.07
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6proR}$	$J_{5,6proS}$	$J_{6proR,6proS}$
	7.0	9.3	9.5	9.5	4.6	1.5	12.3

[a] Solution in $CDCl_3$, Me_4Si as internal reference.

TABLE II

^{13}C Chemical shifts of **1**[a]

C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
100.20	72.20	72.50	68.60	73.55	68.25

[a] Chemical shifts (at 303 K) in p.p.m. relative to internal Me_4Si.

Conformation about the C-5–C-6 bond from the H–H coupling constants. — Measurement of the $^3J_{H\text{-}5,H\text{-}6proR}$ and $^3J_{H\text{-}5,H\text{-}6proS}$ coupling constants provides a way to estimate the rotamer distribution about C-5–C-6 in solution. For this purpose, the following set of equations, established by Manor *et al.*[30] may be used:

$$1.3(gg) + 2.7(gt) + 11.7(tg) = {}^3J_{H\text{-}5,\ H\text{-}6proS}$$

$$1.3(gg) + 11.5(gt) + 5.8(tg) = {}^3J_{H\text{-}5,H\text{-}6proR}$$

$$gg + gt + tg = 1.$$

where *gg*, *gt* and *tg* represent the respective percentage of each conformer. Such a set of equations has been used by Wu *et al.*[31] to investigate the conformational equilibrium of the primary hydroxyl group in methyl pentofuranosides. Nishida *et al.*[32] used the same procedure for hexopyranoses. For the present cyclic peracetylated trisaccharide, straightforward application of the set of equations yields the following results: $gg = 53\%$, $gt = 52\%$, $tg = -5\%$.

It is obvious that the negative population of the *tg* conformer has no physical significance. The conclusion is that both *gg* and *gt* orientations about C-5–C-6 bond are, within experimental error, equally distributed in chloroform-*d* solution.

Estimation of isotropic rotational correlation-time and measurement of proton n.O.e. — The utility of n.m.r. relaxation processes to estimate internuclear distances derives from the possibility of extracting the contribution of the dipole–dipole (*dd*) mechanism from the total relaxation of a nuclear magnetic spin. Dipole–dipole relaxations are dependent on interatomic distances; they are also influenced by motional characteristics of the molecule as characterized by the isotropic rotational correlation-time: τ_c. The sign and maximum theoretical value for any direct n.O.e. is a function of the product $\tau_c\omega_o$ (ω_o being the Larmor frequency). The rotational correlation-time of the sample was estimated from measurements of the carbon–proton spin–lattice relaxation time and carbon–proton n.O.e. For a given proton H_i, the spin–lattice relaxation time T_1 is a function of intramolecular dipole–dipole (*dd*) relaxation, intermolecular (*inter*) relaxation, solvent induced (*solv*) relaxation, and paramagnetic impurities (*para*) and follows the equation:

$$1/(T_1)H_i = 1/(T_1)dd + 1/(T_1)inter + 1/(T_1)solv + 1/(T_1)para \qquad (4)$$

To a good approximation $1/(T_1)H_i = 1/(T_1)dd$. This may be ascertained further by measuring the ^{13}C spin–lattice T_1 relaxation-time as well as the ^{13}C–^1H n.O.e.. These

TABLE III

^{13}C Relaxation time (s) and ^{13}C–^1H n.O.e. values of **1** at two different magnetic fields

	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
T_1 values						
2.35 (*Tesla*)	0.35	0.35	0.35	0.35	0.35	0.18
7.05 (*Tesla*)	0.35	0.35	0.36	0.35	0.35	0.18
n.O.e. values						
2.35 (Tesla)	2.8	3.0	3.0	2.9	2.7	3.0
7.05 (*Tesla*)	2.8	3.0	3.0	3.0	2.8	2.9

measures are summarized in Table III. It is clear that all the carbon atoms have comparable NT_1 values; N being the number of protons attached to each carbon atom. This indicates isotropic motion of the molecule, which tumbles with a correlation time τ_c. From Table III it may be concluded that the n.O.e. effect measured for the carbon atoms is identical, and has a magnitude close to that of the theoretical maximum of 2.99.

$$(\text{n.O.e.})_{obs} = 2.90 \quad (5)$$

$$\eta = (\text{n.O.e.})_{obs} - 1 = 1.90 \quad (6)$$

$$(T_1)dd = (T_1)_{obs} * (1.998/\eta) \quad (7)$$

From equations *6* and *7* the percentage of dipolar relaxation to the total relaxation may be estimated to 95%. The magnitude of the correlation time may be derived from the following equation:

$$(1/(T_1) = R_1 = N_H \hbar^2 (\gamma_C)^2 (\gamma_H)^2 (r_{C\text{-}H})^{-6} \tau_c \quad (8),$$

where a magnitude of 0.10 nm is used for $r_{C\text{-}H}$. The magnitude of the correlation time τ_c is $0.7\ 10^{-10}$s.

The proton n.O.e. were estimated by n.O.e.-difference spectroscopy. The protons H-1, H-3, H-5, H-6pro*R*, and H-6pro*S* were successively saturated and the corresponding n.O.e. difference-spectra allowed calculation of the n.O.e. by measuring the surface area for each protons; all of the measured n.O.e. are reported in Table IV. For each saturated resonance, an intraresidue n.O.e. value was taken as the reference (1.0) and the interresidue n.O.e. values are given relative to this. Such a procedure was designed because relative n.O.e. are less sensitive to a large variety of problems (paramagnetic impurities, concentration of the sample and instrumental conditions) than the absolute ones.

Construction of the cyclic trisaccharide. — On the n.m.r. time-scale the trisaccharide presents three-fold symmetry, and the glucose residues adopt the $^4C_1$ conformation.

TABLE IV

Calculated and experimental relative n.O.e. values for **1**

Proton		*Conformation*[a]			*Exp.*	
		gg	*gt*	*gg*/*gt*	*Rel.*	*Abs.* (%)
	H-3[c]	1.0	1.0	1.0	1.0	23.5
H-1[b]	H-5	0.7	2.3	1.5	1.8	41.5
	H-6proR	0.0	0.0	0.0	0.1	3.3
	H-6proS	0.5	0.0	0.4	0.4	10.1
	H-1	0.9	1.1	1.0	0.7	24.1
H-3[b]	H-5[c]	1.0	1.0	1.0	1.0	33.5
	H-1	0.5	2.5	1.5	1.4	29.3
H-5[b]	H-3[c]	1.0	1.0	1.0	1.0	21.4

[a] *gg*: The n.O.e. values are calculated for the *gg* model of **1**; *gt*: for the *gt* model of **1**; *gg*/*gt*: calculated for **1** for the mixture of *gg* and *gt* model in 1:1 ratio. [b] Indicates the irradiated hydrogen atom. [c] Indicates the hydrogen atom relative to which the n.O.e. values are expressed.

The preliminary calculations were aimed at the construction of starting models to assess whether a cyclic trisaccharide could be built having the six-membered rings in a chair conformation. The conformational investigation of such a cyclic system may be performed by calculating combinations of Φ, Ψ, and ω giving rise to the expected structure and by computing the energy associated with the resulting conformations. By using a rigid geometry for the glucose residues, a set of three glycosidic torsional angles (Φ, Ψ, ω) and the magnitude of the valence glycosidic angle (τ) remain to be determined. A magnitude of $\sim 113°$ may be assumed for this valence angle. Despite the fact that the existence may be assumed of the three stable, non-eclipsing dispositions around the angle ω, values of this angle over the whole angular range, in 30° increments, were considered. For the sake of conciseness, only three potential iso-energy surfaces and the corresponding helical iso-parameters maps ($n = 3$ and $h = 0$) are shown. These maps, which are computed for a disaccharide molecule (gentiobiose), are presented in Figs. 3a, b, and c; they correspond to *gauche-gauche*, *gauche-trans*, and *trans-gauche* orientations, respectively. It may be noted that only two sets of glycosidic torsional-angles can generate a cyclic trisaccharide having low-energy conformations. One corresponds to $\Phi = -80°$, $\Psi = 135°$, $\omega = -60°$, and $\tau = 113.5°$ (model *gg*), and the other to $\Phi = -156°$, $\Psi = 66.5°$, $\omega = 50°$, and $\tau = 114.5°$ (model *gt*). As for a *trans-gauche* orientation about C-5–C-6 ($\omega = 180°$) it is obvious that no cyclization can occur without distorting the $^4C_1$ chair conformation of the glucose residue.

The conformations corresponding to models *gg* and *gt* were used to construct the peracetylated trisaccharides, which were then submitted to complete energy minimization through MM2P-85. The structural features of interest corresponding to the refined geometries have been deposited as supplementary material (see footnote, page 201), along with the energy values. It is noteworthy that there is no significant departure from three-fold symmetry. These two models were considered for the rest of the work and used to model the n.m.r. observations.

Calculated versus observed n.O.e.. — By utilizing the method outlined in the Experimental section, n.O.e. values were calculated for comparison with values determined experimentally. Calculations were performed for each individual model, and for an equiprobable mixture of conformers. These results are reported in Table IV. For each of the irradiated protons, identification of which hydrogen pairs present conformationally independent n.O.e. is quite straightforward. This is particularly useful for scaling the experimentally observed n.O.e. Among the protons, only irradiation of H-3 leads to conformationally independent effects. This is not surprising, as examination of the models indicates that this proton is remote from all of the conformationally sensitive protons of the molecule. Also, the H-3 protons only experience contributions from the H-1, H-2, H-4, and H-5 protons of the same residue. Similar n.O.e. values (upon irradiation of H-3) are calculated for the *gg* and the *gt* model. They exhibit little variation and reflect the subtle differences between the geometry and conformations of each model; they also provide some indication concerning the standard deviations. For this particular proton, there is a good agreement between observed and calculated values for each of the low-energy conformations.

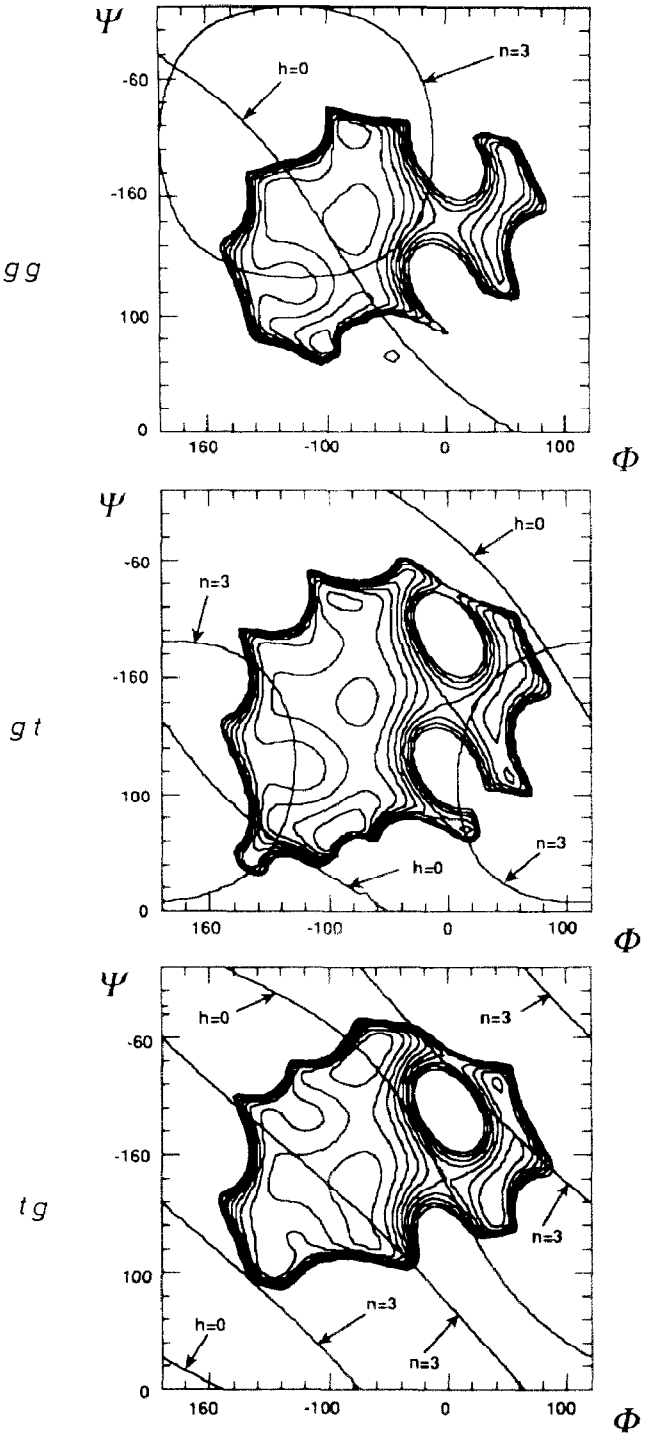


Fig. 3. Iso-energy maps (Φ, Ψ) about the β-(1→6) linkage for gentiobiose and iso-n = 3 and iso-h = 0 contours. The three orientations for the torsion angle ω namely *gauche-gauche* (*gg*), *gauche-trans* (*gt*), and *trans-gauche* (*tg*) are taken into account. For each map, relative iso-energy contours are drawn at intervals of 1 kcal/mol above the minimum.

The H-1–H-5 interactions appear to be very conformationally sensitive, as calculated n.O.e. values for the *gg* and the *gt* model differ strongly. In the *gt* model, the H-5 proton does indeed experience a direct contribution from the irradiated H-1 hydrogens belonging to two consecutive glucose units, B and C, as shown in Fig. 1. The same effect is found for H-1 when H-5 is irradiated, since both H-1A and H-1C are enhanced. None of these strong enhancements are calculated in the case of the *gg* model. As for the H-6pro*R* and H-6pro*S* protons, all of the calculated values indicate a lack of strong enhancement. At this stage it must be stressed that none of the models taken separately can explain the observed n.O.e. values as the experimental values lie between those calculated from the *gg* and *gt* models.

No satisfactory agreement between calculated and observed n.O.e. may be reached without considering an equimolar proportion of the *gg* and *gt* conformers. The choice of this ratio is dictated by the conclusions derived from molecular modelling concerning the sole occurrence of two low-energy conformers, along the with the interpretation of the $^3J_{\text{H-5,H-6pro}R}$ and $^3J_{\text{H-5,H-6pro}S}$ coupling constants. As may be seen from Table IV, the agreement between the calculated and the observed n.O.e.'s of the conformationally sensitive protons is now very satisfactory.

Description of the solution conformations. — The final atomic coordinates corresponding to the two conformations (namely *gg* and *gt*) found in solution have been deposited*. Stereoscopic depictions (PITMOS[33]) of the *gg* conformation and of the *gt* conformation are given in Figs. 4a and 4b, respectively. For the sake of clarity the acetate groups are not shown. As the present results were obtained through a complete geometry-optimization and energy minimization, it would be meaningless to report such stereochemical features as intracyclic bond-lengths and bond-angles. In both conformers the conformation of the glucopyranose ring is $^4C_1$, whereas the secondary acetate groups are arranged in such a way that the carbonyl nearly eclipses the axial hydrogen atom at the corresponding ring-carbon atoms. There is no significant departure away from the usual $^4C_1$ conformation. The stereochemical features occurring at the glycosidic linkages (Φ, Ψ, ω, and τ) have been deposited as supplementary material. There is no noticeable departure from three-fold symmetry in either conformation. In both conformers the acetate groups point away from the molecule and therefore they do not perturb the establishment of the conformations at the glycosidic linkages.

For the *gg* conformation, the glycosidic torsion angles average $\Phi = -79.5°$, $\Psi = 143.5°$, and $\omega = -64.3°$, respectively. The average value of the valence angle τ is 113.5°. (These values should be compared with those derived from the crystal structure of gentiobiose[34] $\Phi = -58.3°$, $\Psi = -156.3°$, $\omega = -61.5°$, and $\tau = 113.3°$). This conformation corresponds to an orientation of Φ in agreement with the establishment of the exoanomeric effect. As for the *gt* conformation, the glycosidic torsion-angles average $\Phi = -137.7°$, $\Psi = 68.2°$, and $\omega = 45.6°$, respectively. The average value of the

* The deposited materials may be obtained from Elsevier Science Publishers B.V., BBA Data Deposition, P.O. Box 1527, Amsterdam, The Netherlands. Reference should be made to No. BBA/DD/457/*Carbohydr. Res.*, 211 (1991) 191–205.

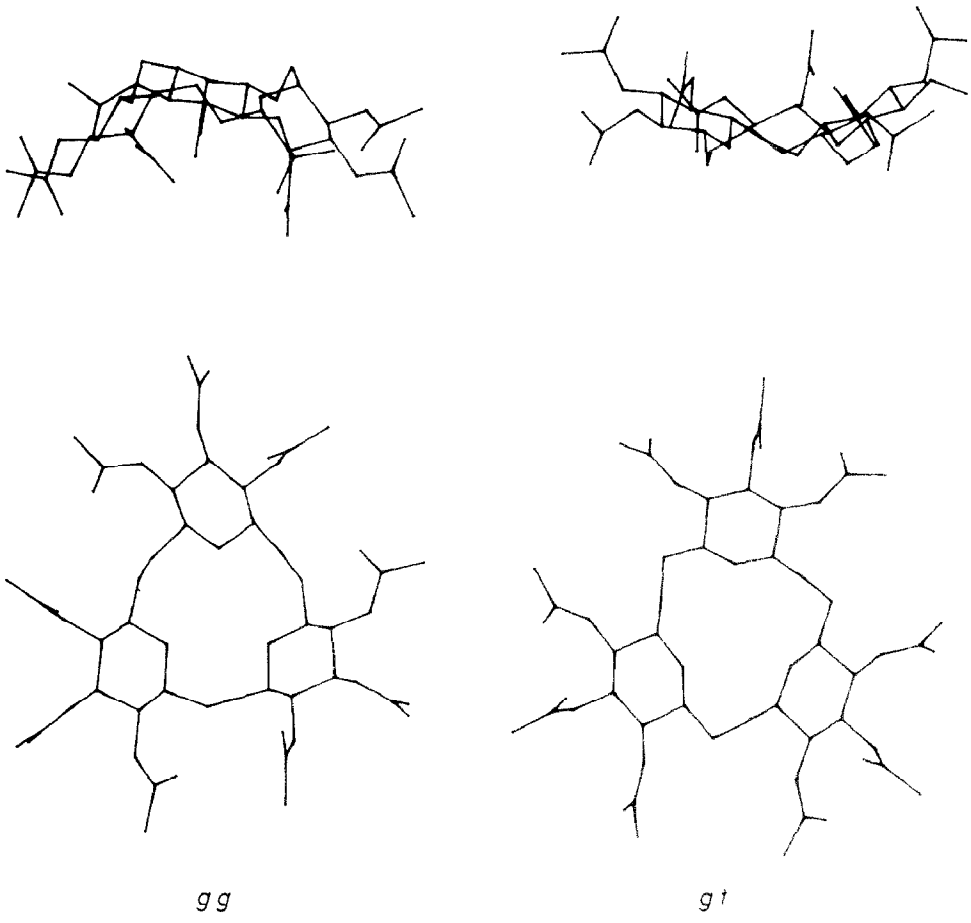


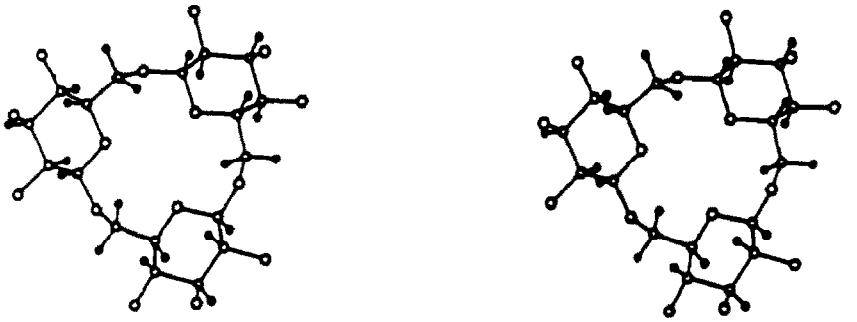
Fig. 4. Molecular representations of the two stable conformations of [O-β-D-glucopyranosyl-(1→6)]-1,6″-anhydride nonaacetate: (*a*) *gg* model, (*b*) *gt* model.

valence angle τ is 115.5°. The existence of this conformer shows that conformational populations are not dominated by the stabilizing influence of the exoanomeric effect.

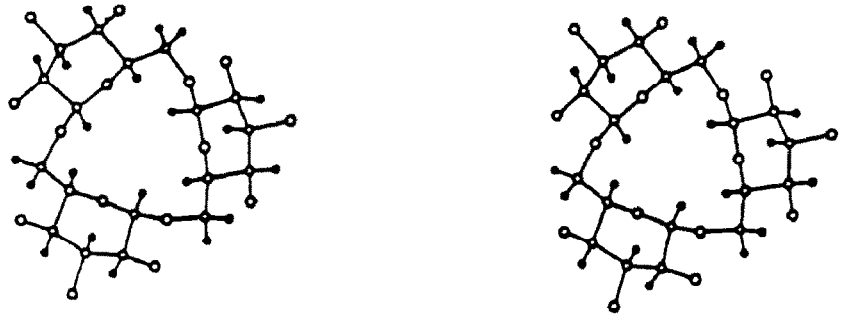
Both conformers exhibit a torus-like shape, 16 Å at its maximum extension and ~6 Å in width. The polar part of it corresponds to the regular alternation of ring oxygen and glycosidic oxygen atoms; it is located almost at the bottom of the cavity, which has a width of ~4 Å. It is difficult to assess whether the cavity is accessible to a molecular host for interactions. The bottom of this cavity is protected from interaction by a hydrophobic layer made up of hydrogen atoms.

A striking difference between the two conformers is that they display opposite curving; whereas the *gg* conformer has a concave shape, the *gt* conformer has a convex surface (Fig. 5).

Discussion of the significance of the energy difference between the stable conformers. — Interpretation of the coupling constants and n.O.e. leads unequivocally to the



gg



gt

Fig. 5. Molecular representations showing the opposite curving displayed by the *gg* (concave shape) and *gt* (convex shape) models.

conclusion that two conformations occur equally in solution. However, quite a different conclusion would be reached had the conformer population been estimated from a Boltzman distribution based on energy differences. In the MM2P-85 scheme, conformer *gg* has an energy of 102.3 kcal/mol, whereas conformer *gt* has an energy of 114.4 kcal/mol. The strict application of Boltzman distribution would give a very small population to the *gt* conformer in a vacuum. Such a discrepancy could result from inadequate parameterization of the energy calculations in MM2. It is clear that the existence of a stable conformation having Φ about $-140°$ (very different from the one predicted from the exoanomeric effect) is never experienced in crystal structures, and therefore may be missing in the set of observed data used in the parameterizations. Moreover, such a conformation is not considered stable in some of the semi-empirical quantum-mechanical treatments such as MNDO (Modified Neglect of Differential Orbitals), whereas it corresponds to a stable state according to such other methods as PCILO (Perturbative Configuration Interaction with Localized Orbitals) (I. Tvaroska

and J. P. Carver, personal communication). Before drawing any conclusion about the shortcomings of intramolecular energy-calculations, several points should also be examined. (*a*) Clearly, the omission of the solvation contribution to the energy may bias the estimation of relative population of the two conformers, which would interconvert in solution. (*b*) None of the conclusions that are drawn can distinguish between a situation where the two conformers are interconverting in solution and one where two independent and separate conformers are present. The first hypothesis, which implies a fast rearrangement in solution from one conformer to the other, would occur following a conformational pathway involving a cooperative deformation of the $^4C_1$ chair conformation of the glucose residues. It is possible that low-temperature studies would lead to coalescence and resolved signals for the two "conformers", confirming the fast-exchange hypothesis. The application of molecular dynamics to this problem would provide some detailed indications about the conformational transitions. The second hypothesis implies that, when the internal cyclization of the bifunctionalized linear trisaccharide takes place, two different structures differing only in their conformations at the glycosidic linkages are formed in similar proportions. However, the n.m.r. spectrum is compatible with a unique structure, suggesting that, if two structures coexist in solution, all their n.m.r. parameters are identical. This is highly improbable. Until this problem is solved, no definite conclusion about the deficiency of intramolecular energy-calculations, if any, can be made.

CONCLUSIONS

One of the major conclusions of this work is that the normal n.m.r. spectrum alone cannot establish whether a carbohydrate in solution exists as a "single" conformer or whether several conformers are present in fast exchange. This distinction emerges only later, during the interpretation of the n.O.e. data, which assesses the relative populations of the conformers. It appears that the computational scheme to evaluate n.O.e. (and T_1) data, as pionnered by Carver and co-workers[3,3b], is sound. A similar conclusion may be drawn for the utilization of $^3J_{H,H}$ coupling constants in the assessment of conformer population about C-5–C-6 bonds. The present example represents a particularly favorable case for conformational analysis in that equilibrium may be described using just two well-defined conformations. Surprisingly, in spite of this, quantitation of conformer population from energy calculations has proved difficult. Several explanations which require further investigations are proposed.

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