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THE MULTIPLE CONFORMATIONS OF A CYCLIC DISACCHARIDE: DI-β-D-GLUCOPYRANOSE 1,6′:1′,6-DIANHYDRIDE HEXAACETATE*

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ABSTRACT

The conformational behaviour of a cyclic disaccharide, di-β-D-glucopyranose 1,6′:1′,6-dianhydride hexaacetate, has been investigated. Because this molecule can exist only with the glucose rings in the unusual flexible forms, such conformational parameters as pseudorotation phase-angles have been used. Within a given number of approximations, the conformational space available for the whole system can be explored by considering only one two-dimensional map. Detailed investigations have shown that three stable conformations may be proposed. Among these, two correspond to minima found in the solid state. In one form, the six-membered rings adopt a boat conformation, whereas a skew conformation is found for the other form. However, these two conformations cannot be considered to be unique models of the conformation in solution; they both produce sets of proton–proton coupling-constants inconsistent with observed n.m.r.-spectroscopic results. At least the third form, having the six-membered rings in skew conformations, has to be taken into account. Deviations from coupling constants–molecular conformation relationships are thought to originate from ring strain.

INTRODUCTION

In the course of polycondensation of 2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosyl bromide[1], a side-reaction of internal cyclization yielded the peracetylated disaccharide: di-β-D-glucopyranose 1′,6:1′,6-dianhydride hexaacetate (**1**). Its ^{1}H- and ^{13}C-n.m.r. spectra show that the two symmetrical parts of the molecule are magnetically equivalent[2]. A preliminary conformational investigation[3] predicted a 2,9,15,16-tetraoxatricyclo[9.3.1.1.^{4,8}]hexadecane backbone-structure having a strained conformation in which the D-glucopyranose

*Dedicated to Professor Stephen J. Angyal on the occasion of his retirement.

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rings do not adopt a normal, stable chair-form. X-Ray structural elucidation[4] indicates that **1** crystallizes in the triclinic P_1 space group, with two independent molecules in the unit cell. The main structural features evident from this work are the following. The internal cyclization yields unusual strain and conformational distortion in the D-glucopyranose rings. The strain is located essentially about the endocyclic C–O–C bond angles, whose mean values are up to 8° larger than those usually found in strainless pyranoid rings. Notable conformational differences between the two independent molecules are found. In one molecule, the six-membered rings approximate to a boat conformation, whereas in the other, a skew conformation exists. For both molecules, there exists a pseudo-axis of symmetry. Unusual conformations are also observed for the ten-membered rings.

Compound **1** thus offers a unique instance of a strained molecule in which conformational flexibility seems to be accommodated. Moreover, the glucopyranose residues adopt unusual conformations. Therefore, a detailed study of such a molecular system appeared warranted. Conventional strategy for conformational calculations with oligo- and poly-saccharides of D-glucose starts with the hexose residues kept in the usual, stable $^4C_1$ form; rotations about the glycosidic torsion-angles are considered as the main contributors to the overall energy. A totally different strategy has to be followed for dealing with such systems as the cyclic disaccharide **1**. First of all, conformational parameters relevant for the description have to be proposed, and the conformationally allowed regions have to be investigated as a function of these parameters. Only in the last steps of the work may detailed energy-calculations be performed. The application of such a scheme for study of compound **1** is described here.

METHODS AND PROCEDURES

Conformational parameters. — A (1→6)-glycosidic linkage between residues j and $j + 1$ offers three contiguous torsion-angles, ϕ, ψ, and Ω, where:

$$\phi = (O_j\text{-}5\text{–}C_j\text{-}1\text{–}O_j\text{-}1\text{–}C_{j+1}\text{-}6),$$
$$\psi = (C_j\text{-}1\text{–}O_j\text{-}1\text{–}C_{j+1}\text{-}6\text{–}C_{j+1}\text{-}5),$$
and $$\Omega = (O_j\text{-}1\text{–}C_{j+1}\text{-}6\text{–}C_{j+1}\text{-}5\text{–}O_{j+1}\text{-}5).$$

Despite the degree of flexibility offered by three contiguous torsion-angles, no cyclic disaccharide can be constructed having the six-membered rings in a chair conformation[5]. Therefore, flexible forms of the glucopyranose residue must be invoked, and parameters relevant for such a description have to be found.

Conventional energy-calculations would necessitate consideration of numerous parameters such as torsion angles, and would make any rationalization of the conformational behaviour of the cyclic disaccharide impossible. For simplification, the following approximations were made: (*a*) the acetate groups were not considered; (*b*) the glucose residues were considered as cyclohexane, assuming all bond-lengths to be 1.54 Å, and the hydrogen-atom positions used were computed according to a

perfect tetrahedral arrangement, with a value of 1.09 Å being assigned to C–H distances; and (*c*) for the ten-membered ring, such approximations as fixed bond-lengths (1.54 Å) and bond-angles (109.47°), as well as the occurrence of a C_2 axis of symmetry, were made.

The flexible conformations of such an idealized glucose residue may be investigated by a method comparable to that proposed for similar studies with cyclohexane[6-8]. Bearing in mind that all bond-lengths and bond-angles are kept invariant, all flexible conformations of the six-membered ring may be described by the torsion angles θ_1, θ_2, and θ_3 (see Fig. 1). Instead of considering these three torsion-angles, a pseudo-

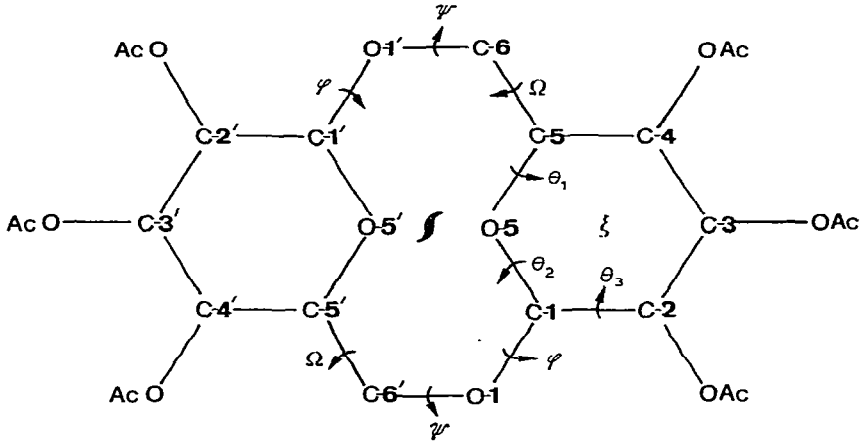

Fig. 1. Schematic diagram of di-β-D-glucopyranose 1,6′:6,1′-dianhydride hexaacetate (**1**) and the relevant torsion-angles. The symbol ∫ indicates the C_2 axis of symmetry within the molecule.

rotation phase-angle ξ, varying from 0 to 360°, may be used. The angle ξ allows for a backward determination of θ_1, θ_2, and θ_3 through the use of a function $f(\xi)$ of the form: $\theta_1 = f(\xi)$; $\theta_2 = f(\xi - 120)$; and $\theta_3 = f(\xi + 120)$. Throughout this work the following function was used:

$$\theta_1 = C_1\sin(\xi + \xi_0) + C_2\cos(\xi + \xi_0),$$

where $C_1 = 69.282$ and $C_2 = 0.025$ (ref. 3) and ξ_0 has an arbitrary value corresponding to the pseudorotation phase-origin. The flexible forms of the glucose residue are depicted in Fig. 2, in which the origin of the pseudorotation phase has been set to the $^{2,5}B$ conformation[9].

It may readily be visualized from Fig. 1 that a set of only two parameters (ξ,Ω) permits complete description of the conformation of the cyclic disaccharide exhibiting a perfect C_2 axis of symmetry. Nevertheless, in order to account for the cyclic nature of the disaccharide under study, another condition must be introduced; this can be performed by assigning a given value to the glycosidic bond-angles; for example, C-6–O-1′–C-1′. For a given value of the glycosidic bond-angles and a given set of (ξ,Ω) values, there corresponds a finite number (0, 1, 2) of valid solutions. When the

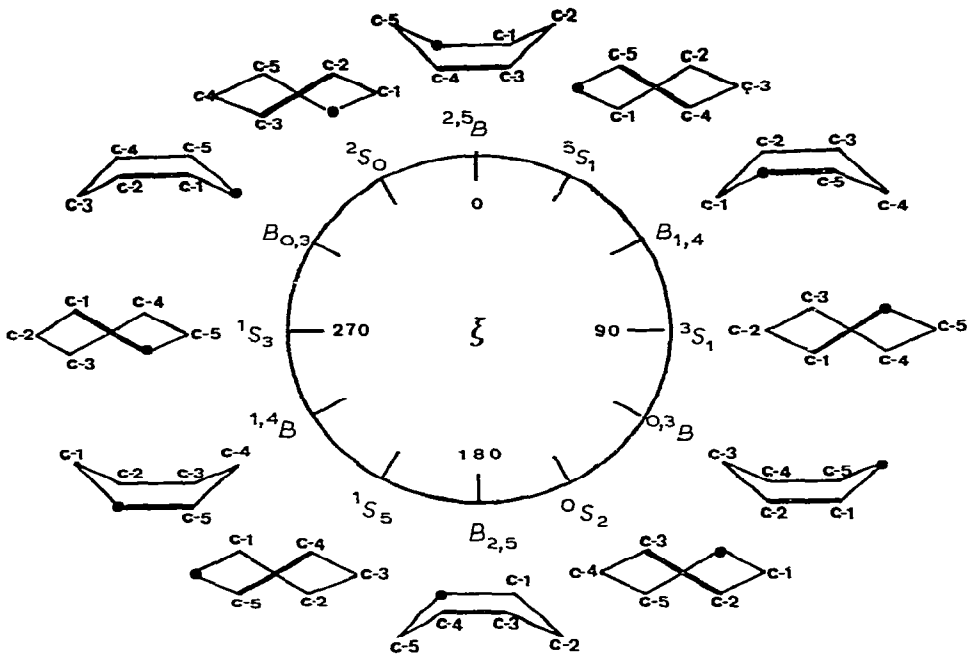

Fig. 2. Flexible forms of the D-glucopyranose ring[9]. The pseudorotation phase-angle ξ has been set to the $^{2,5}B$ conformer. Black circles in the pyranoid rings denote oxygen atoms.

number of solutions amounts to two, these latter differ only by the value of the torsion angle ψ. As the value of the glycosidic bond-angles is kept constant, the conformational space of the whole system may be explored by considering at most two (ξ,Ω) maps.

Conformationally allowed regions. — Considering the number of approximations necessary to undertake study of the cyclic disaccharide, the criteria for energy evaluation should remain as simple as possible, at least in the first step. At this stage, the bond lengths and bond angles are kept invariant. Moreover, as the precise energy of the flexible forms of the glucose residues has not been evaluated, energy arising from torsional potential along the ten-membered ring was also neglected. Within these approximations, the contributors to the difference between conformations described by (ξ,Ω) are steric interactions. For any type of interaction, a single cut-off distance was selected. Two calculations were performed, one corresponding to a cut-off distance of 2.0 Å, the other to 1.6 Å. These distances are significantly lower than the sum of the van der Waals radii for H, C, and O atoms. The use of such cut-off distances can be justified on the basis that obvious inaccuracy would result from the number of approximations made, unless a severe criterion is introduced that would act as a strong discriminator. As such, these values for the cut-off distance may not have any profound physical meaning.

By using this rather crude approach, the conformationally allowed regions of the disaccharide were investigated in terms of (ξ,Ω); the results are depicted in Fig. 3. There is one set of ψ solutions that yields an area compatible with the selected criteria.

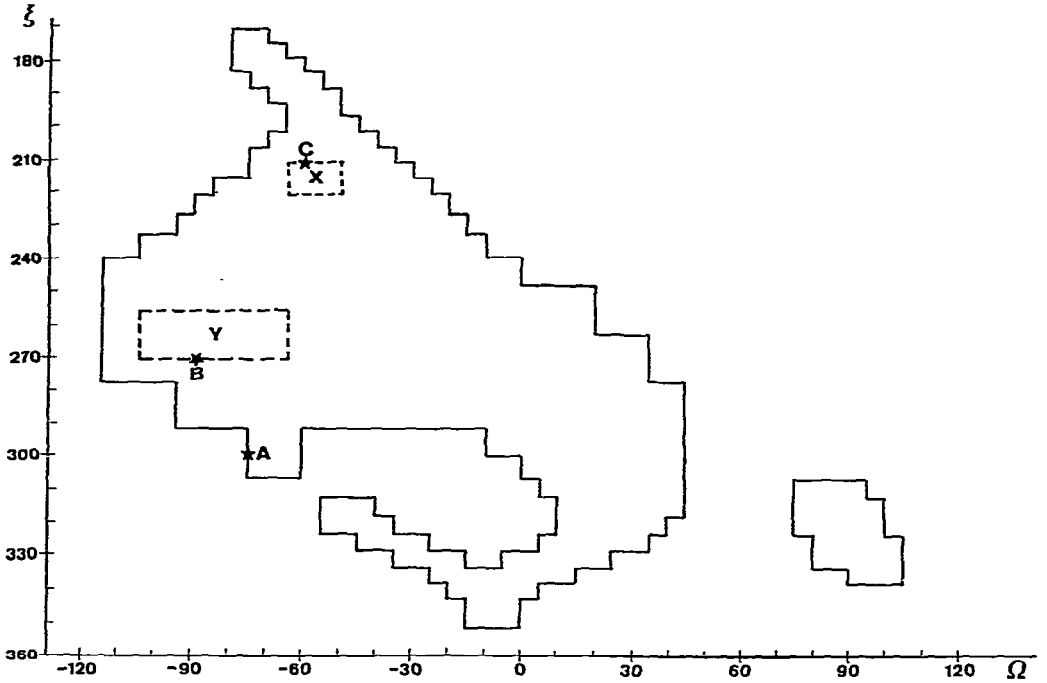

Fig. 3. Conformationally allowed regions for the cyclic disaccharide **1** as a function of ξ and Ω (ξ is given the same origin as in Fig. 2). Combinations of ξ and Ω compatible with the 2.0-Å and 1.6-Å cut-off distance criterion are shown as broken lines (– – –) and solid lines (——), respectively. For molecules A and B, the pseudorotation phase-angles best describing the averaged conformations of the glucose residues are indicated, together with the values. The refined conformation C is also shown.

The other set of ψ solutions corresponds to situations where the molecule collides with itself. Therefore, the available space for the disaccharide is entirely accessible on only one map, expressed as a function of (ξ,Ω). By using the 2-Å distance criterion, only two allowed regions are found (designated as X and Y in Fig. 3). With the 1.6-Å distance criterion, the available space is extended and corresponds to conformations where flexible forms, varying from $B_{2,5}$ to $^2S_0$, can be accommodated, with Ω values in the range —110 to 40°.

Conformational minima. — Experimental results from the X-ray study may be expressed in terms of the conformational parameters ξ and Ω. In the following presentation, the two experimentally observed molecules are designated A and B. Figs. 4a and 4b show views of the backbone conformations of A and B, respectively. It may be seen that C_2 symmetry is almost perfectly achieved. Therefore, this symmetry is imposed on any further calculations for molecules A and B. For molecules A and B, the pseudorotation phase-angles ξ best describing the averaged conformations of the glucose residues, together with the Ω torsion angles, are (300°, —76°) and (273°, —87°), respectively. The observed crystallographic minimum B (273°, —87°) lies very close to one of the two calculated minima (Y) obtained with a 2-Å distance criterion; the glucose residues are in a skew conformation. The second crystallo-

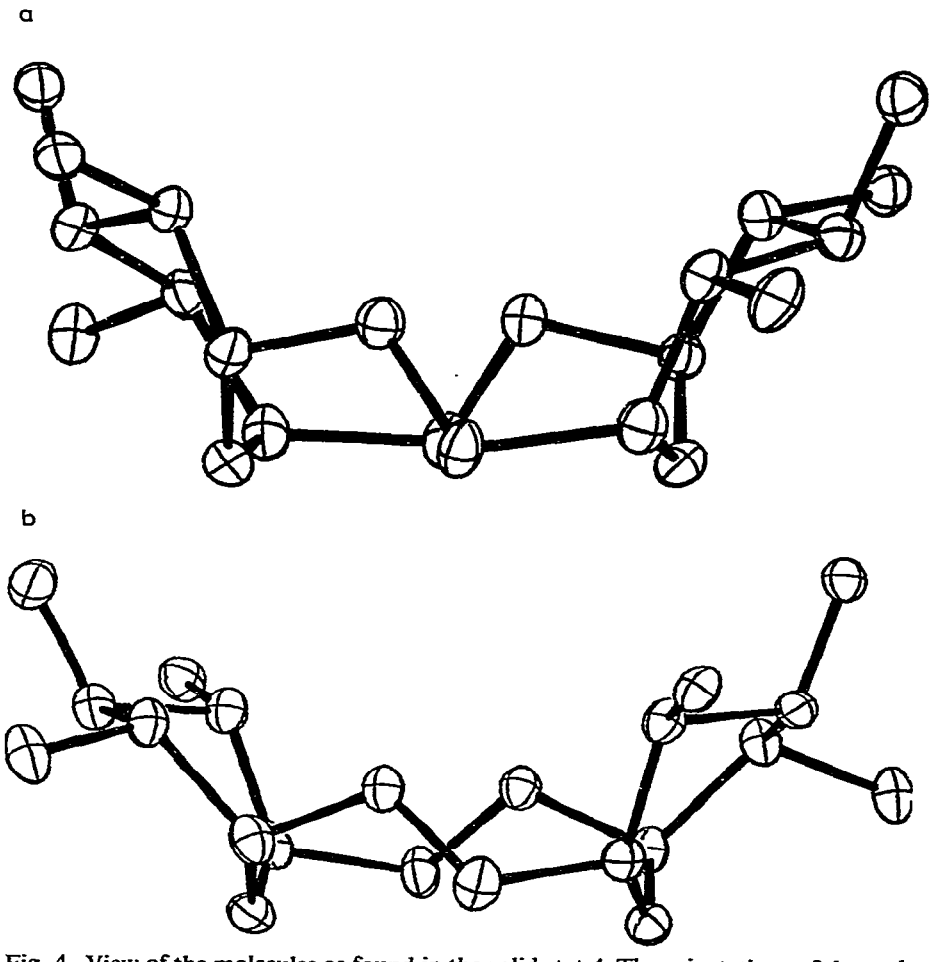

Fig. 4. View of the molecules as found in the solid state[4]. The orientations of the molecules are such that the almost perfect symmetry may clearly be seen. The pendant acetate groups have been omitted. In (a), molecule A, the glucose residues have a boat conformation, and in (b), molecule B, they have a skew conformation.

graphic minimum obtained, A (300°, −76°), is located at the periphery of the map (Fig. 3) that was calculated with the 1.6-Å criterion; the glucose residues have a boat conformation. From this result it may be concluded that the foregoing theoretical treatment permits a reasonable understanding of the conformational behaviour of the cyclic disaccharide **1**.

Among the probable conformations (Fig. 3), it is interesting that one, namely X, is not found in the solid state. Therefore, in order to corroborate the probable or improbable occurrence of this form, a more elaborate conformational analysis was undertaken. The calculations made use of semi-empirical functions that consider bond lengths and deformations of bond angles, as well as torsional potentials. Nonbonded interactions were evaluated by using Hill-type functions[5,10]. The con-

straint of C_2 symmetry within the molecule was still assumed. The minimization was performed by using a Simplex type of procedure[11]; the starting values of the minimized parameters were close to those defining the idealized form corresponding to $\xi = 210°$ and $\Omega = -60°$. This calculation led to prediction of a stable conformation where the main contributors to the energy can be subdivided as follows: (*a*) 1% from deformation of bond lengths (0.2 kcal.mol^{-1}); (*b*) 28% from deformation of bond angles, located essentially about the C–O–C angles (4 kcal.mol^{-1}); (*c*) 56% from rotations about the torsional angles, located essentially within the flexible glucopyranose-rings (8 kcal.mol^{-1}); and (*d*) 15% from nonbonded interactions (2.1 kcal.mol^{-1}).

In this conformation, the glucopyranose rings are found in a skew conformation. The overall conformation of the backbone of the molecule, designated C, is given in Fig. 5.

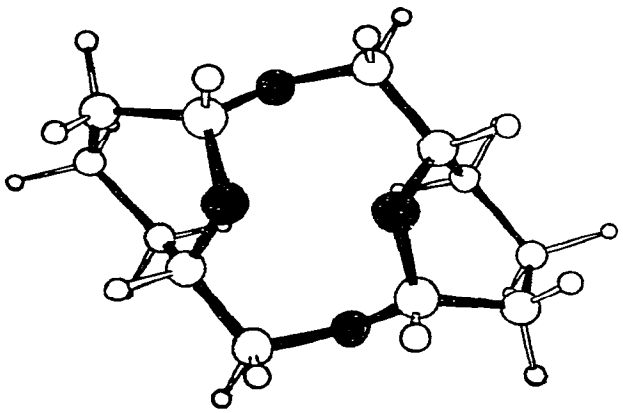

Fig. 5. View of the molecule C as found from conformational energy-calculations. The pendant acetate groups have been omitted. The glucose residues have a skew conformation.

In order to compare the relative stability of the molecules as found in the solid state with respect to that derived from the foregoing considerations, conformational analysis of forms A and B was undertaken. This was performed by using the same set of conformational parameters; the starting values of the minimized parameters were close to those defining the idealized forms of A and B, namely ($\xi = 300$, $\Omega = -76°$) and ($\xi = 273°$, $\Omega = -87°$), respectively. The conformationally refined parameters showed good agreement with those found from X-ray data. This finding supports the correctness of our calculations. In terms of total energy involved in each form, it was shown that A, B, and C could be assigned very comparable values. Conformations B and C have identical energies, and form A is only 0.5 kcal.mol^{-1} higher in energy than the others. Moreover, close examination of the influence of the different contributors to the energy of A and B yielded values of the same order of magnitude as those already mentioned. The main geometrical parameters describing conformer C are given in Figs. 6a and 6b, together with those corresponding to the

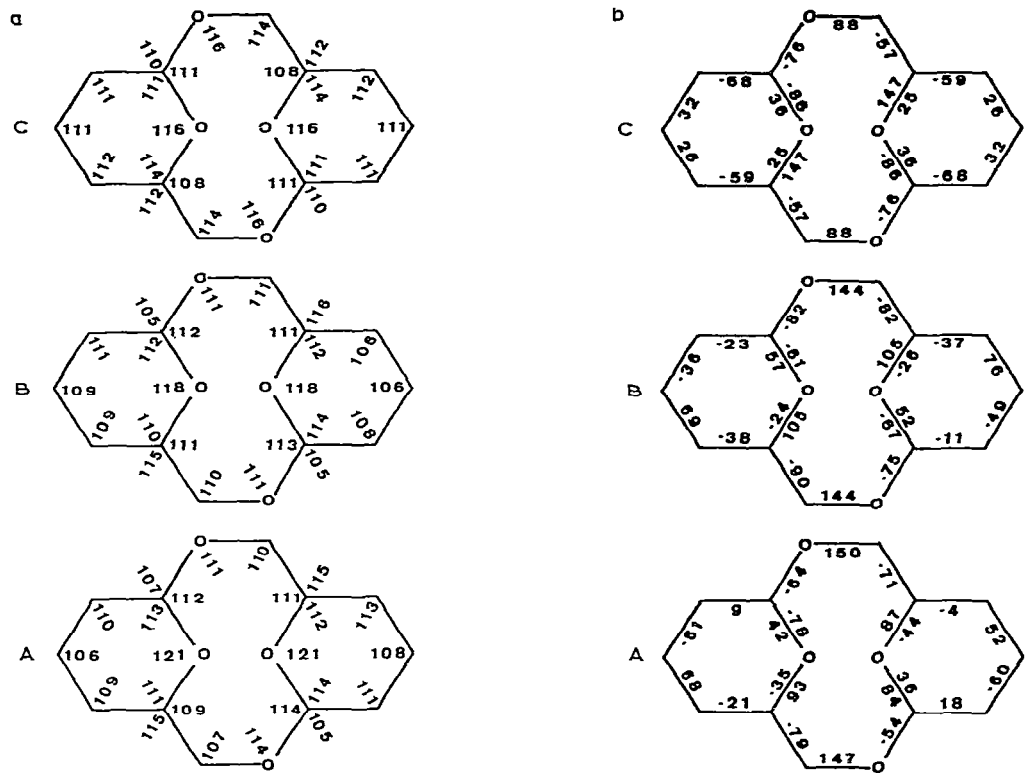

Fig. 6. Comparison between the calculated conformation C and the observed molecules A and B, as found in the solid state[4]. Model C was calculated by assuming C_2 symmetry within the molecule. (a) Bond angles; (b) torsion angles.

experimentally observed, crystallographic conformations. It is noteworthy that, on the (ξ,Ω) map, the three minima A, B, and C are associated with a column centered around $\Omega = -60°$; the main differences occurring from the pseudorotation phase-angle ξ.

DISCUSSION

The results presented here show that, within the carbohydrate field, flexible forms of glucopyranose rings may be encountered and can lead to conformational isomerism for the same molecule, even in the solid state. Thus far, such behaviour has generally been considered common only for furanoid rings. Deviation from the stable chair-conformation of glucopyranose rings has been invoked only in the case of the chitohexaose-lysozyme complex. In the work described here, flexible forms of the glucopyranose residues are induced by a high degree of intramolecular strain. If a similar level of strain could be induced by intermolecular forces, the foregoing theoretical treatment, making use of pseudorotation phase-angles, should be con-

sidered to be a method for conformational investigation of the resulting flexible system.

The values of torsion angles defining the disaccharide backbone are of obvious interest, from the n.m.r.-spectroscopic viewpoint, as they correspond to values uncommon for carbohydrate structures. Comparison of the observed coupling constants with values expected from the torsion angles, through use of Karplus relationships, would enhance the scope of correlations existing between coupling constants and molecular structures. Prerequisite to such a study is a qualitative agreement between n.m.r. results and the proposed, stable conformations. The ^{1}H- and ^{13}C-n.m.r. spectra, and also the coupling constants calculated from a second-order analysis of the 100-MHz ^{1}H spectra, have already been reported[1].

The proton–proton coupling-constants, together with the values of the relevant torsion-angles as found in molecules A, B, and C, are schematically depicted in Fig. 7. It is clear that neither the crystallographic conformations A or B, nor the calculated form C, may be considered individually as valid models. They all produce sets of coupling constants inconsistent with observed values. Furthermore, if the three conformations are considered equiprobable and are given a weighting factor of 1/3, the set calculated is still inconsistent. The results may be analyzed in more detail as follows. For molecules A, B, and C, the conformations about C-5–C-6 are rather similar; they give results consistent with the experimentally observed $J_{5,6}$ and $J_{5,6'}$ coupling constants. Indeed, the values of the torsion angles are close to those usually found in aldohexopyranoses[1,2], and no strain is observed in this part of the

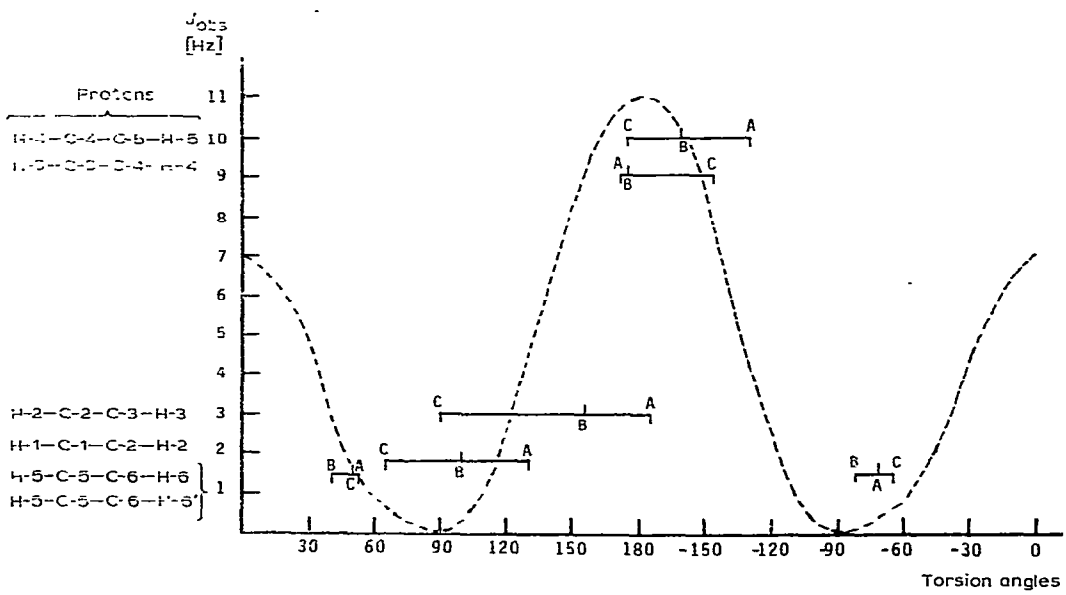

Fig. 7. The values of the torsion angles as found in A, B, and C are plotted as a function of the observed proton–proton coupling-constants[2]. Attention is drawn to the range of variation exhibited by some of the torsion angles. The dotted line helps to display the general trend of the Karplus plot and thereby show the inadequacy of conformations A, B, and C in correlating the entire set of observed couplings.

molecule. On the other hand, very large differences between values about comparable torsion-angles are observed in the strained, flexible part of the molecule. For example, conformations about C-2–C-3 span over 90°, and here no agreement can be reached. Such failure most probably arises from drastic deviations, in rings where strain exists, away from conventional coupling-constants–molecular conformation relationships. The occurrence of stable conformations different from that studied may also be invoked.

CONCLUSIONS

Study of the cyclic disaccharide described in this work shows that a strained molecule may nevertheless exhibit multi-conformational behaviour. Such a finding, which is quite unusual for glucopyranose residues, has been investigated in detail by use of such conformational parameters as pseudorotation phase-angles associated with the flexible forms of the glucose residues. More-detailed calculations have shown that the two conformations found in the solid state can be assigned comparable energy. Furthermore, another stable conformation exhibiting the same level of calculated energy is proposed. As such, this cyclic disaccharide appears to be an excellent example for use as a standard for testing methods or empirical sets of parameters for energy calculations.

Concerning the proton–proton coupling-constants, the present example shows that conformations found in the solid state should not be considered to be the only models for conformation in solution. Other forms, such as the one derived from conformational calculations, should also be taken into account. However, no valid relationships between coupling constants and conformation have been found for this molecule, a discrepancy that may be attributed to the high degree of strain in this cyclic disaccharide.

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