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CONFORMATIONS OF DISACCHARIDES BY EMPIRICAL FORCE-FIELD CALCULATIONS PART II, β-CELLOBIOSE

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ABSTRACT

Conformations of β-cellobiose have been studied by using convergent energy minimisation in a simple force field Parameters for the force field were taken from similar studies on α- and β-D-glucopyranose and β-maltose Six local minima are found on the ϕ,ψ-map, the free enthalpy differences are 1 4, 7 5, 8 5, 10 2, and 30 1 kJ mol^{-1} above the lowest, corresponding to a distribution of 60 34 3.2 1 0 at 298 K Each of these minima is surrounded by a manifold of minimum conformers that differ only in exocyclic torsions Conformations of the two lowest minima are close to X-ray structures The path on the ϕ,ψ-map of conformational interchange between the two lowest minima has been investigated, and barrier height and rates of conversion have been estimated Fast conversion between the lowest minima is supported by optical rotation measurements

INTRODUCTION

In the previous paper[1] of this series, conformations and conformational interchange of β-maltose were described We now present a similar conformational analysis of β-cellobiose, through energy minimisation in which all internal degrees of freedom are allowed to relax

Nomenclature In the following, β-cellobiose shall mean 4-*O*-β-D-glucopyranosyl-β-D-glucopyranose The constitution of β-cellobiose, giving atom numbering and the torsional angles ϕ and ψ for later reference, is shown in Fig 1, ϕ and ψ are defined by atoms H(C-1′)–C-1′–O-4–C-4 and H(C-4)–C-4–O-4–C-1′, respectively Our definition of a torsional angle follows the IUPAC convention[2] A torsional angle A–B–C–D is defined through a Newman projection, it is the angle through which A–B must be rotated around B–C to cover C–D when looking from B towards C, the sign is positive if the sense of rotation is clockwise

Earlier calculations. Empirical calculations of the potential energy of non-bonded interactions indicate that β-cellobiose has a number of conformations which

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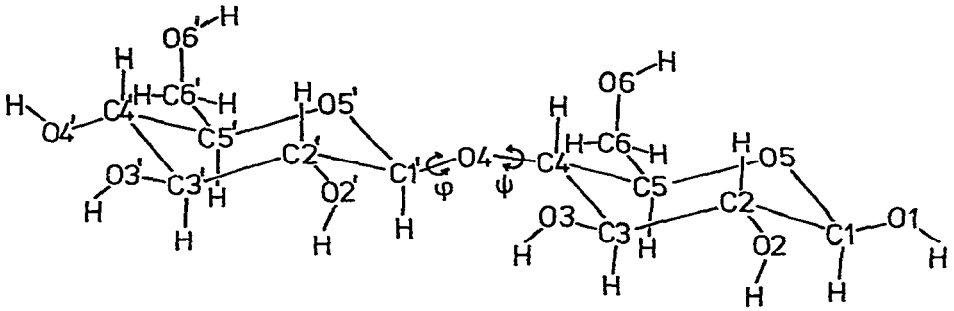

Fig 1 Constitution and atom numbering for β-cellobiose.

correspond to minima on the potential-energy surface Rees and Skerrett[3], selecting one out of three different types of potential-energy functions for the non-bonded interactions, found minima at $(\phi,\psi) = (0, -37)$ and $(41, -5)$ when "Kitaygorodsky type functions" were used The relative energy difference between the two minima was $\sim 0\,4$ kJ mol^{-1}

Semi-empirical calculations (the PCILO method) have been done by Giacomini *et al*[4] They found minima at $(\phi,\psi) = (94, -22)$, $(23,-44)$, $(98,-24)$, $(41,-84)$, and $(78,-174)$, corresponding to energies of $-12\,0$, $-10\,9$, $-8\,4$, $-0\,8$, and $0\,8$ kJ mol^{-1}, respectively

During recent years, Rees and Smith[5], in an empirical framework, found a minimum for β-cellobiose at $(\phi,\psi) = (30,-25)$

The above-mentioned calculations have a common feature in that no energy minimisation was performed, they are simply mappings over the two-dimensional ϕ,ψ-space

Experimental studies Three X-ray structures are relevant for comparison with calculated conformations of β-cellobiose The fractional co-ordinates taken from these X-ray diffraction structures were transformed to cartesians, from which we calculated the internal co-ordinates The structure of β-cellobiose has been determined several times[6-8], and the most-refined data are those of Brown[7] ($R = 0\,059$) and Chu and Jeffrey[8] ($R = 0\,037$), values for (ϕ,ψ) and the glycosidic angle C-1′–O-4–C-4 were $(44,-12)$ and $116\,7°$, and $(42,-18)$ and $116\,1°$, respectively The structure of methyl β-cellobioside methanolate[9] ($R = 0\,060$) was transformed as mentioned above, but the z co-ordinates were changed to $-z$ in order to get the D forms of the pyranose rings, (ϕ,ψ) was $(25, -48)$ and C-1′–O-4–C-4 was 115.8°.

N m r studies, using vicinal ^{13}C–^{1}H coupling-constants, indicate that the conformation of cellobiose in aqueous solution is close to that in the solid state[10] The same conclusion was derived from optical rotation studies of cellobiose[11]

The ^{1}H-n m r spectrum of cellobiose in methyl sulphoxide[12] shows that the intramolecular hydrogen bond O-3–O-5′ which is observed in the crystal structures[6-8] is probably not broken in solution, and it seems that ϕ and ψ are mainly determined by intramolecular forces This is parallel to the conclusions drawn for β-maltose[1]

CALCULATIONS

The computational methods and programmes used here were developed from the CFF system of Lifson and Warshel[13] by Niketić and Rasmussen[14] The main difference between our calculations and the earlier ones is that we perform energy minimisation, which means that all degrees of freedom are allowed to relax, *i e*, no internal co-ordinate is kept fixed We then relate minima on the potential-energy surface to equilibrium conformations

Force field In previous work, we developed a force field for α- and β-D-glucopyranose[15,16], and we found it satisfactory for calculation on β-maltose[1] It is a very simple and conventional force-field, which uses harmonic functions for bond and angle deformations, Pitzer terms for torsional motions, and Buckingham potentials for non-bonded interactions All interactions separated by three or more bonds are considered non-bonded

$$\text{Bond deformations} \quad E_b = \sum_{\text{bonds}} \tfrac{1}{2}K_b(b - b_o)^2$$

$$\text{Valence-angle deformations} \quad E_\theta = \sum_{\text{angles}} \tfrac{1}{2}K_\theta(\theta - \theta_o)^2$$

$$\text{Torsional deformations} \quad E_\phi = \sum_{\text{torsions}} \tfrac{1}{2}K_\phi(1 + \cos 3\phi)$$

$$\text{Non-bonded interactions} \quad E_{nb} = \sum_{i>j} \{A_{ij} \exp(-B_{ij} r_{ij}) - C_{ij}/r_{ij}^6\}$$

The 36 parameters needed for these potential-energy functions are given in our previous work[1]

Initial conformations All initial conformations of β-cellobiose, where nothing else is stated, were produced by suitable rotations around the bonds of the glycosidic linkage of the minimised crystal-structure conformation[8] We selected a total of 22 points on the φ,ψ-map

Energy minimisation All initial conformations produced in this way had a fairly high energy-gradient and some of them were far from a minimum The gradient was lowered very efficiently by a steepest descent algorithm followed by a modified Newton algorithm[14] The same strategy for minimisation as described in our previous paper[1] was used for β-cellobiose Minimisation was considered finished when the norm of the gradient became less than 10^{-6} kJ mol^{-1} Å^{-1}

Conformational interchange The modified Newton algorithm is designed to follow the bottom of a valley on the potential-energy surface, and this feature makes it suitable in a search for saddle points It is thus possible to find relevant paths on the φ,ψ-map for the conformational interchanges between two minima

RESULTS AND DISCUSSION

The minima Using the experience gained from calculations on β-maltose, and

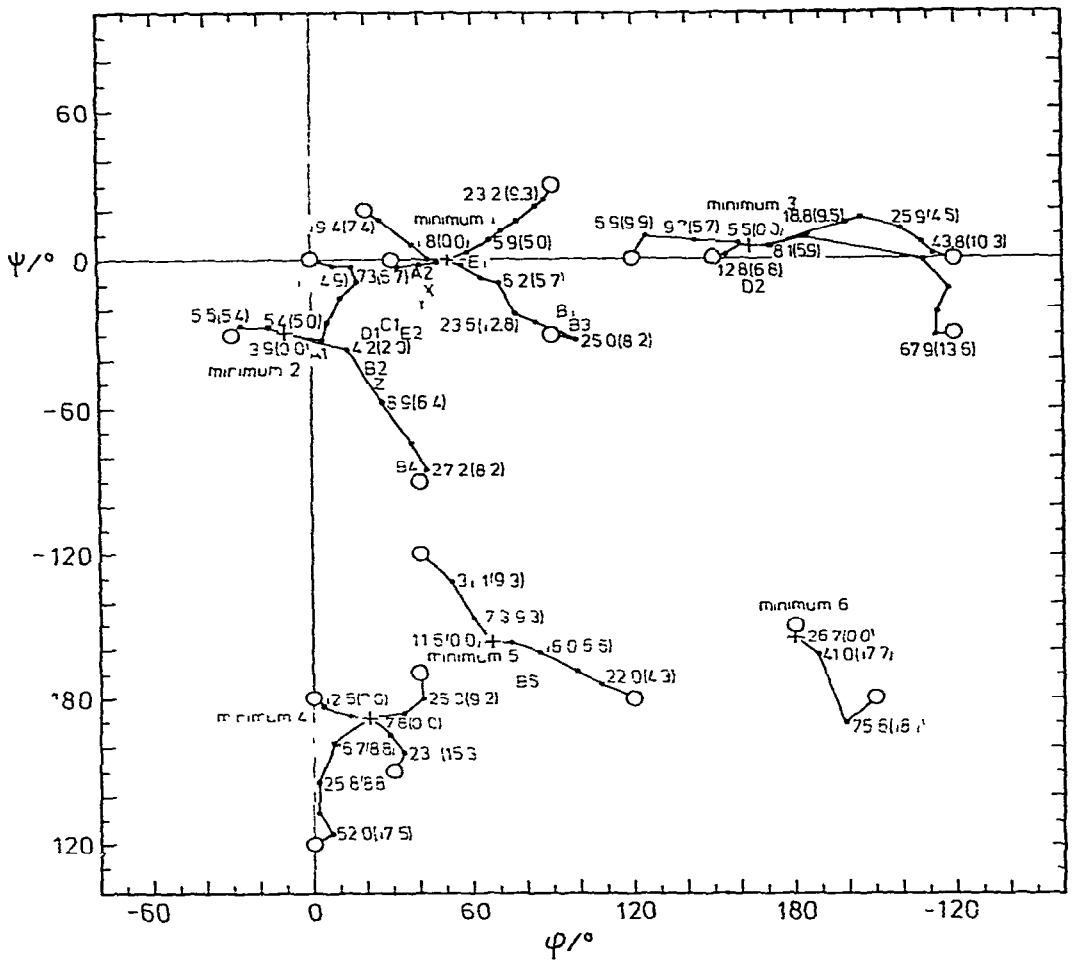

Fig 2 Initial conformations (○) and final minima (+) of β-cellobiose on a ϕ,ψ-map Total energy in kJ mol^{-1} and energy-gradient norm in kJ mol^{-1} Å^{-1} (in parentheses) of intermediate and minimum conformations Results of Rees and Skerrett[3] (A1–A2) Giacomini *et al*[4] (B1–B5), Rees and Smith[5] (C1), Rees and Skerrett[18] (D1–D2), and Sarko[19] (E1–E2) X-Ray results of Brown[7] (X), Chu and Jeffrey[8] (Y), and Ham and Williams[9] (Z)

chemical intuition together with molecular models, it was possible to exclude a great number of initial conformations as irrelevant

All of the relevant initial conformations are pictured on a ϕ,ψ-map in Fig. 2. From this, it is seen that all the initial conformers were minimised to six different local minima that are listed in Table I.

The potential surface as presented on the ϕ,ψ-map consists of two valleys located approximately at $\psi = 0°$ and $\psi = 180°$, separated by high mountain ranges The valley containing minima 1, 2, and 3 is rather flat, meaning that the energy barriers between these minima are low (see below) Minima 4, 5, and 6, on the other hand, are separated by high energy barriers In this connection, it should be mentioned that minimum 6, although energetically unfavourable, is not an artifact in the same

TABLE I

GLYCOSIDIC TORSIONAL AND VALENCE ANGLES, FREE ENTHALPY (T = 298 K), TOTAL ENERGY, AND ENERGY CONTRIBUTIONS FOR SIX EQUILIBRIUM CONFORMERS OF β-CELLOBIOSE

Minimum	ϕ	ψ	*C-1'–O-4–C-4*	G_o	E_T	E_b	E_θ	E_ϕ	E_{nb}
	(degrees)			*(kJ mol^{-1})*					
1	51	0	113 4	881 35	1 82	2 42	6 58	0 05	−7 23
2	−10	−29	114 4	882 75	3 94	2 48	7 93	0 06	−6 53
3	164	5	115 7	888 81	5 47	2 83	10 38	0 05	−7 79
4	21	172	116 1	889 80	7 82	2 97	12 48	0 04	−7 67
5	67	−157	117 0	891 53	11 63	3 05	14 13	0 03	−5 58
6	−179	−155	120 8	911 42	26 74	3 70	27 92	0 03	−4 91

sense as minimum 5 of maltose[1] Changing the parameters K_θ(C–O–C) from 602 1 to 209 2 kJ mol^{-1} rad^{-2} does not have any significant influence on the ϕ,ψ-position of the six minima

Evaluating statistical sums over all of the internal degrees of freedom[17], we find at 298 K an equilibrium distribution based on differences in free enthalpy of minimum 1 minimum 2 minimum 3 minimum 4 minimum 5 minimum 6 = 60 34 3 2 1 0

As pointed out for maltose[1], we do not attempt to find the global minimum, which would mean minimising a vast number of initial conformations differing only in torsions around exocyclic C–O bonds Therefore, in this work, we present only the skeletal minima, because it is known[1 15] that with any local energy minimum there is associated a manifold of minimum conformations corresponding to different values of exocyclic torsional angles Concerning the two -CH_2OH groups we have adopted the geometrical positions from the crystal structures[7,8]

Comparing Fig 2 with Fig 2 of our previous paper[1], it is seen that the main features of the potential surface are repeated, particularly the minima located in the area around (ϕ,ψ) = (0,0) Because of the opposite absolute configuration at the anomeric carbon atom of the non-reducing D-glucosyl group, this does not mean that the structures of the two disaccharides are very much alike This has consequences for the polymers Thus, amylose, an α-linked polymer of D-glucose, has a helical structure, whereas cellulose, which is β-linked, is an extended-chain polymer

Comparison with crystal-structure data Table II shows a comparison of our calculated internal co-ordinates for minimum 1 with the X-ray data from the crystal-structure determination of β-cellobiose[8] Deviations (calculated minus measured values) of bond lengths and angles are shown. For bond lengths and valence angles, maximum, mean, and mean square-root deviations are listed these internal co-ordinates are reproduced very satisfactorily, as well as those reported earlier[1 15,16] For endocyclic (containing only ring atoms) and hybrid torsions (containing three

TABLE II

DEVIATIONS OF CALCULATED FROM MEASURED[8] INTERNAL CO-ORDINATES FOR MINIMUM 1 OF β-CELLOBIOSE

	Maximum	*Mean*	*Mean square-root*
Bond lengths, Å	0 043	0 006	0 015
Valence angles, °	−3 3	−0 1	1 8
Endocyclic torsions °	6 2		3 8
Hybrid torsions, °	8 8		5 4
	$\lvert\phi_{calc} - \phi_{meas}\rvert$		
Exocyclic torsions, °			
C-4–C-5–C-6–O-6	9 6		
C-4′–C-5′–C-6′–O-6′	9 6		
Intercyclic torsions, °			
O-5′–C-1′–O-4–C-4	6 5		
C-3–C-4–O-4–C-1′	13 7		

ring atoms and one side-atom), maximum and mean square-root deviations are listed they are slightly larger than in the case of β-maltose The deviations for two exocyclic (containing two ring atoms and two side-atoms) and the two intercyclic torsions (corresponding to ϕ and ψ) are also shown

In addition, for minimum 1, we calculate the glycosidic angle C-1′–O-4–C-4 and the two endocyclic valence angles, C-5–O-5–C-1 and C-5′–O-5′–C-1′ to be 113 4, 112 6, and 112 6°, respectively From the X-ray data[8], these angles were 116 1, 113 5, and 112 5°

Comparison with earlier calculations In addition to our results, Fig 2 shows all equilibrium conformers found by mapping in a semi-empirical or empirical framework as mentioned in the Introduction In addition, a few minima for cellulose, found by empirical work[3 18 19], are included It is seen that the present work not only includes the minima of the previous investigations but also specifies new ones (minima 4 and 6) The potential-energy surface for β-cellobiose shown in Fig 2 is, to a first approximation, very similar to those given by Giacomini *et al*[4] and Rees and Smith[5] for cellobiose, and Rees and Skerrett[3 18] and Sarko [19] for cellulose

A more-careful comparison of the minima shows that the PCILO calculation[4] gave minimum conformations far from our minima, only one of the five PCILO minima was in the region around minima 1 and 2 On the other hand, the empirical calculations on cellobiose[3,5,18] and on cellulose[3,18,19] found minima near our minima 1, 2, and 3 and the saddle point (see below)

Path of conformational interchange. As mentioned already, our modified Newton algorithm can be used to explore the potential-energy surface. In this way, we can find the way in which a conformational change between two equilibrium conformers

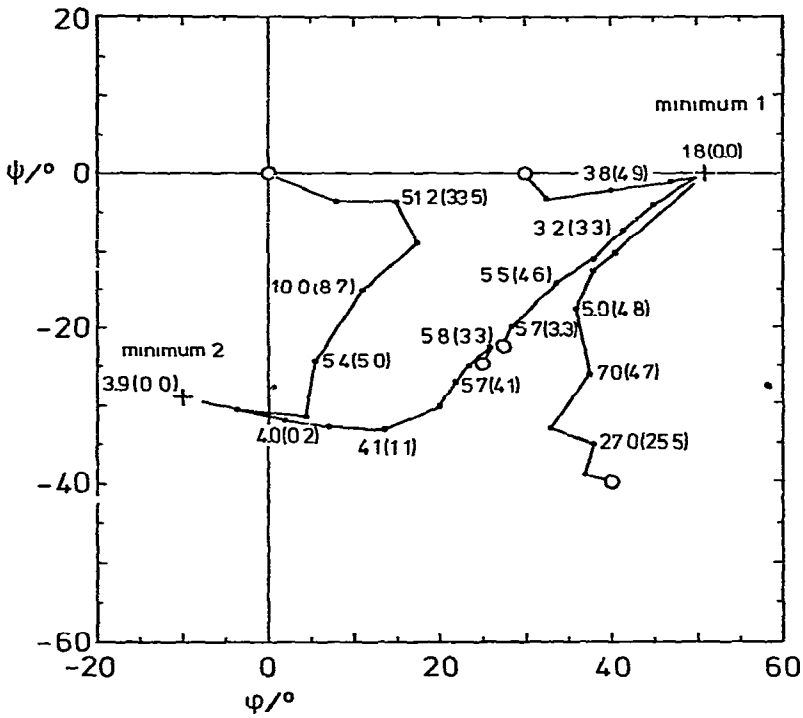

Fig 3 ϕ,ψ-map of β-cellobiose around minima 1 and 2 Total energy in kJ mol^{-1} and energy-gradient norm in kJ mol^{-1} Å^{-1} (in parentheses) of intermediate and minimum conformations The saddle-point conformation is located around $(\phi,\psi) = (26, -23)$

may occur in the chosen force-field Here, we shall be concerned with the path for conformational change between minima 1 and 2

Fig 2 gives some idea of the appearance of the potential surface, and Fig 3 shows a section of the ϕ,ψ-map in the region containing these two minima together with a few more minimisations From the calculations, it was evident that the path of interchange from minimum 1 to minimum 2 must be something like a change in both ϕ and ψ from (51,0) to about (20,—30), followed by a change in ϕ from 20° to —10° with ψ kept fixed at —30° In Fig 4, ORTEP stereo-drawings of minimum 1, the approximate saddle-point conformation, and minimum 2 are shown

Since the form of the potential surface is very sensitive to the energy parameters, the height of the barrier between the two minima of Fig 3 should be taken as only an approximation to the real barrier. Within this limitation, the barrier height from minimum 1 to minimum 2 may be given as ~3 kJ mol^{-1} and in the reverse direction as ~2 kJ mol^{-1}.

Consequences of conformational interchange From these barriers, the rates of conformational interchange between minima 1 and 2 may be calculated, assuming an Arrhenius expression with a frequency factor corresponding to a glycosidic torsional frequency of the order of 100 cm^{-1}

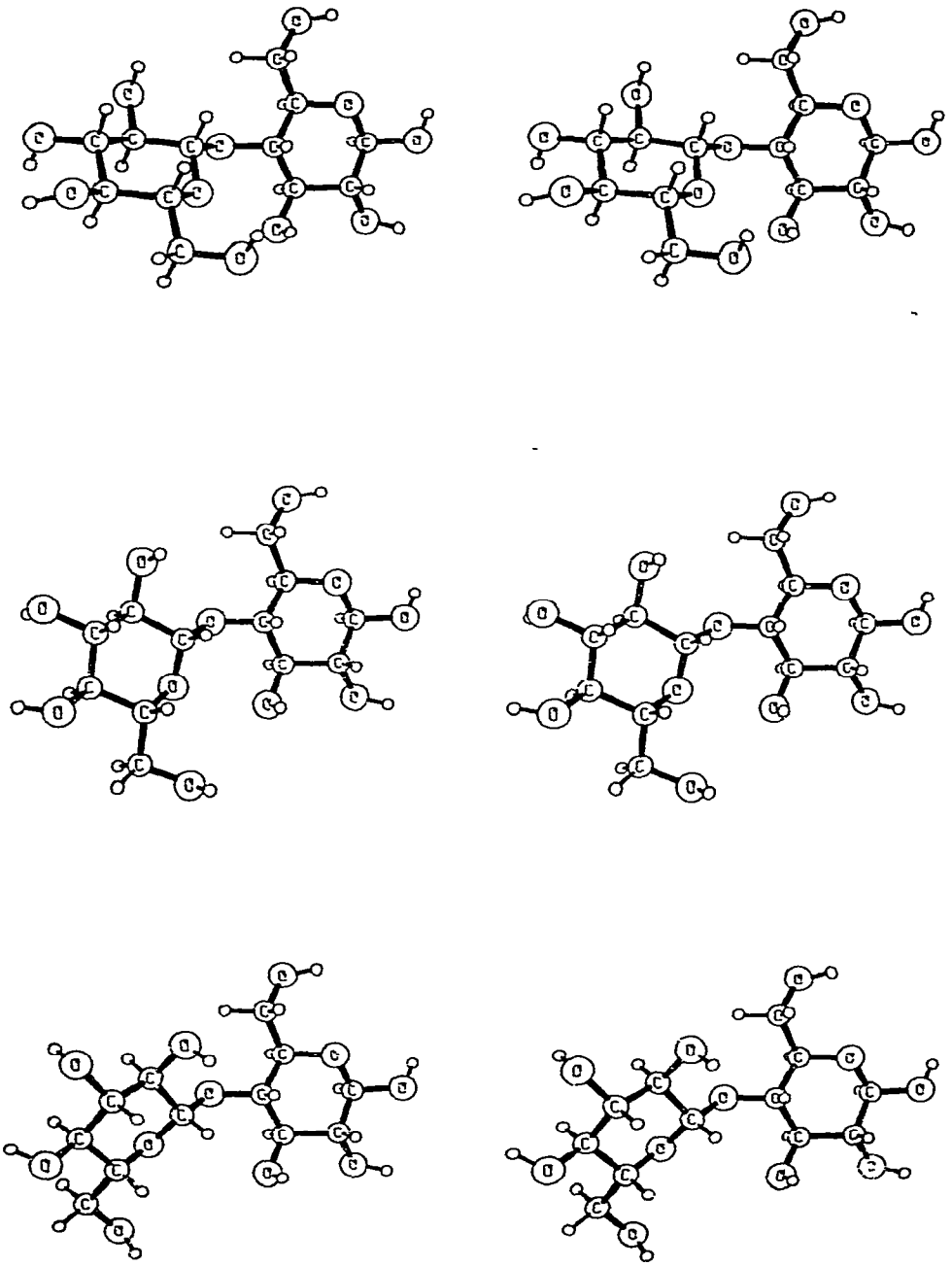

Fig 4 ORTEP stereo-drawings of minimum 1, (ϕ,ψ) = (51,0) (upper), approximate saddle-point conformation, (ϕ,ψ) = (26, −23) (middle), and minimum 2, (ϕ,ψ) = (−10, −29) (lower)

$$k_{12} = 100\,cm^{-1} \cdot 3 \times 10^{8}\,cm\,sec^{-1} \quad exp(-3000/8\,3143/298) \approx 9\ GHz,$$
$$k_{21} \approx 13\ GHz$$

Such rates, comparable to those found for β-maltose, are fast on the n m r time-scale, being of about the same order of magnitude as the molecular tumbling or probably even faster Therefore, the conformations of β-cellobiose which can be derived from n m r and other measurements must be weighted averages of, in principle, six (in practice, maybe essentially only three) equilibrium conformations, modified by thermal vibrations

As mentioned before, experimental studies on cellobiose show that the conformation in solution is very similar to that in the crystal structure[11 12] Furthermore, Rees and Thorn[20] found that the optical rotation was dependent on solvent and temperature, indicating that cellobiose in solution is not locked in the crystal conformation but oscillates in the neighbourhood of this structure. This conclusion is in good agreement with what is found for the interchange between minima 1 and 2

The distance between H(C-1′) and H(C-4) was 2 29, 2 16, 3 52, 3 57, 3 56, and 4 01 Å for minima 1–6, respectively The weighted average, using the distribution calculated earlier, is 2 32 Å If, because of the high ridges, only minima 1, 2, and 3 are considered, the average is 2 28 Å, which is hardly a significant difference*

Vibrational spectra would be superpositions of the spectra of the species present Normal co-ordinate calculations[14] show that those spectra are very much alike, and a distinction would be impossible

Material available Internal and cartesian co-ordinates, and stereo drawings, of all equilibrium conformers may be obtained from the authors

CONCLUSION

In a previous paper[1], we described how it is possible, given proper minimisation techniques and a reasonable force-field, to calculate realistic structures for such flexible molecules as disaccharides, exemplified by β-maltose

For β-cellobiose, we now find six local minima on the ϕ,ψ-map, each of which is surrounded by a manifold of minimum conformers that differ only in exocyclic torsions

The multidimensional energy-hypersurface is explored, predicting intramolecular dynamics in the given force-field The path on the ϕ,ψ-map for conformational interchange between minima 1 and 2 is given, together with approximate barrier-heights These are very low, because of change in all internal co-ordinates in the saddle-point conformation This means that those non-bonded interactions that would cause a very high and unfavourable, non-bonded energy contribution, if bond lengths and valence angles were kept fixed, can change in such a way that E_b and E_θ will increase slightly while a larger increase in E_{nb} is avoided

* Proton relaxation measurements give results[21] of 2 1–2 2 Å

Our results on β-cellobiose are in agreement with the available, experimental evidence and can be used as a guide in explaining the different structures of cellulose

The entire work on β-maltose and β-cellobiose cost less than 8 h on an IBM 370/165, including all minimisations, vibrational analyses, and thermodynamic calculations

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