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CONFORMATIONAL STATES OF CELLOBIOSE AND MALTOSE IN SOLUTION: A COMPARISON OF CALCULATED AND EXPERIMENTAL DATA

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ABSTRACT

A theoretical conformational analysis of the methyl β-glycosides of cellobiose and maltose was carried out and average values of the coupling constants $^3J_{C\text{-}1,H\text{-}4'}$ and $^3J_{C\text{-}4',H\text{-}1}$ and optical linkage rotation (Λ) were calculated. It was shown that, in aqueous solutions of the disaccharide derivatives, there was a complex conformational equilibrium. The above values fitted the experimental data well if they were obtained by taking into account non-bonded interactions and torsion energy. The exo-anomeric effect and intramolecular hydrogen-bonding in aqueous solutions were shown to be of no significance.

INTRODUCTION

Structural studies of disaccharide units are an important preliminary to the elucidation of the spatial arrangement of oligo- and poly-saccharide chains. The correctness of the empirical calculation of conformations using atom–atom potential functions depends largely upon the choice of the parameters. Comparison of the potential functions proposed by various authors, as exemplified by calculations of disaccharide conformations, were previously made, for example, by Rees and Skerrett[1] who used the then limited data on X-ray structures of oligosaccharides as a criterion. The question as to which of these parameters is applicable for predicting oligosaccharide conformation, especially in aqueous solution, remains undecided. The present investigation is an attempt to resolve this question.

Cellobiose, maltose, and their methyl β-glycosides are convenient models for such an investigation, since the coupling constants $^3J_{C,H}$ defined by the torsion angles about the glycosidic bond are known from the ^{13}C-n.m.r. spectra[2–4]. Also available are the optical rotations for disaccharides[5,6] defined by the conformational state of the "linkage fragment" C-1–O–C-4′.

Our task was to reproduce the values observed for disaccharides by calculations taking into account the total potential-energy surface using various parameters for the force field. Such a comparison enables the distribution of conformations of disaccharides in solution to be described reasonably.

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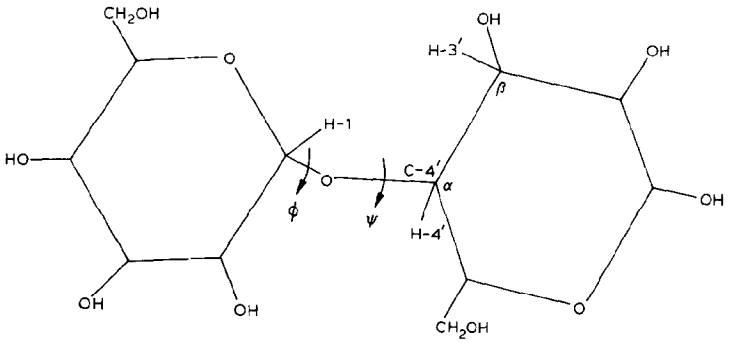

Fig. 1. Molecular model of cellobiose in the conformation ϕ 30°, ψ 175°.

The conformations of (1→4)-linked disaccharides are defined by the torsional angles about the glycosidic bond C-1–O (ϕ) and O–C-4′ (ψ); ϕ is zero when the C-1–H-1 and O–C-4′ bonds in the fragment H-1–C-1–O–C-4′ are *cis*, whereas ψ is zero when the C-1–O and C-4–H-4′ bonds in the fragment C-1 –O–C-4′–H-4′ are *cis* (Fig. 1). A clockwise rotation, viewed from C-1 to the linkage oxygen and from the latter to C-4′, is considered to be positive.

In evaluating the potential energy of conformers, account was taken of non-bonded and electrostatic interactions, torsional energy, and the formation of intramolecular hydrogen bonds. The significance of the exo-anomeric effect for the final results was also investigated.

In order to evaluate the dependence of the results of the calculation upon the choice of atom-atom potential functions, the parameters most commonly applied[7–9] in the conformational analysis of biopolymers were used. The barriers of rotation in the Pitzer potential $U_{tors} = U_o/2(1 + \cos 3\phi)$ around the bonds C-1–O and O–C-4′ were assigned[10] a value of 0.9 kcal/mol.

The electrostatic interactions and energies of hydrogen bonds are sensitive to the nature of the solvent. These energy constituents were approximated for an aqueous medium, and the electrostatic interaction energy was estimated with a monopole approximation (for the charges of a carbohydrate residue's atoms, see ref. 11) and an effective dielectric permeability of 10. This value of ε_{ef} is most appropriate, for example, in considering conformations of dipeptides and polypeptides in aqueous solution[12,13].

The energy parametrisation for hydrogen bonds OH···O, taken into account by using the Morse potential[13], is a much more complicated problem, since no precise data for aqueous solution are available. Since the energies of hydrogen bonds OH···O and NH···O in non-polar solvents are similar[14], they were assumed to be similar in aqueous solution. The energy of interpeptide hydrogen bonds NH···O in water has been estimated[15,16] to be 0 or −1.5 kcal/mol. Therefore, the energy parameter for optimal hydrogen-bonding OH···O in the Morse potential was varied: namely, 0, −1, and −2 kcal/mol.

Recently, among the factors influencing the conformation of carbohydrate

chains, much attention has been paid to the exo-anomeric effect. The angle ϕ also defines the relative position of the lone pairs of electrons on O-5 and O-1 of the non-reducing residue. Their interaction is minimal when the aglycon carbon (C-4′) is *gauche* to the C-1–O-5 bond and maximal when it is *trans*. Lemieux[17] took into account, in addition to the non-bonded interactions (with Kitaigorodsky's parametrisation[9]), the dependence of the exo-anomeric effect on the torsion angle ϕ, obtained from vacuum quantum-mechanical calculations for dimethoxymethane[18]. In this case, the difference between electron energies of *gauche* and *trans* conformers was found to be 3–5 kcal/mol. In order to evaluate the significance of the HSEA method, calculations were made for disaccharide conformations based on the parametrisation of the force field given in ref. 19. However, we argue that the results of vacuum calculations for dimethoxymethane are inapplicable to carbohydrate chains, especially for aqueous media. Thus, for example, the anomeric effect, basically identical to the exo-anomeric effect, for glucose[20] in aqueous solution is only 0.55 kcal/mol.

We have analysed the potential surfaces of disaccharides by several theoretical approximations. The dependence of the shape of the conformational map upon the parametrisation of non-bonded interaction functions was studied first. Allowance was then made for intramolecular hydrogen-bonding and for the exo-anomeric effect.

Consider first the optical rotation data bearing on the conformations of disaccharides in solutions. The linkage rotation (Λ) is a special component of the molecular rotation of disaccharides. The value of Λ is directly related to the conformational parameters ϕ and ψ by the equations[5] $\Lambda_\alpha = -105 - 120 (\sin \phi + \sin \psi)$ and $\Lambda_\beta = 105 - 120 (\sin \phi + \sin \psi)$ (degrees) for α- and β-linked disaccharides, respectively. From these equations, individual conformer rotations $\Lambda^{\phi,\psi}$ may be determined; then, using Boltzmann's probabilities of conformers, the average linkage rotation value $\langle\Lambda\rangle$ may be calculated from the following equation:

$$\langle\Lambda\rangle = \frac{\sum_\phi \sum_\psi \Lambda^{\phi,\psi} e^{-\Delta U^{\phi,\psi}/kT}}{\sum_\phi \sum_\psi e^{-\Delta U^{\phi,\psi}/kT}}.$$

Experimentally obtained Λ values for aqueous solutions of the disaccharides under study are given in refs. 5 and 6.

Vicinal coupling constants depend upon a single conformational parameter, and, for disaccharides, the torsional angles ϕ and ψ, which are defined by H-1–C-1–O–C-4′ and C-1–O–C-4′–H-4′, respectively, may be deduced from carbon–proton (and also carbon–carbon) coupling constants. These constants for all the disaccharides in question, in solution in D_2O, were determined from ^{13}C-n.m.r. spectra[2–4]. For the sake of convenience, J^ϕ and J^ψ are used to connote $^3J_{C\text{-}4',H\text{-}1}$ and $^3J_{C\text{-}1,H\text{-}4'}$, respectively. The dependence of J^ϕ and J^ψ on the torsional angles ϕ and ψ, as proposed by Perlin *et al.*[3], was used for the determination of J^ϕ and J^ψ of individual conformers. The mean values of $\langle J^\phi\rangle$ and $\langle J^\psi\rangle$ (Hz) were determined for the confor-

mational map of each disaccharide drawn for the torsional angles changed by increments of 10° (an increment of 5° did not change the results of statistical analysis).

For conformational calculations, it is necessary to know the spatial structures of monosaccharide residues. Since most of the experimental data discussed below were obtained for methyl β-glycosyl-glycosides, spatial structures of disaccharides based on the results of X-ray investigation of methyl β-D-glucopyranoside[21] for the reducing residue were constructed. For the non-reducing residues, the results obtained for methyl β-D-glucopyranoside[21] and methyl α-D-glucopyranoside[22] were used. From the statistical treatment of the structural data[23], the glycosidic valence angle was assigned a value of 117°. Furthermore, the effect of varying this angle from 113 to 119° was investigated; the optimal value was 117°.

RESULTS AND DISCUSSION

Methyl β-cellobioside. — Conformational maps ϕ (C-1–O) − ψ (O–C-4′) are shown in Fig. 2; Fig. 2a takes into account the non-bonded interactions (functions of Scott and Scheraga) as well as electrostatic and torsional interactions, and Fig. 2b also includes the formation of intramolecular hydrogen bonds. Four local minima associated with optimal conformers are positioned on the potential surface of the disaccharide; their energetic and geometrical parameters are summarised in Table I. Conformers A and B possess the lowest energy of non-bonded interactions,

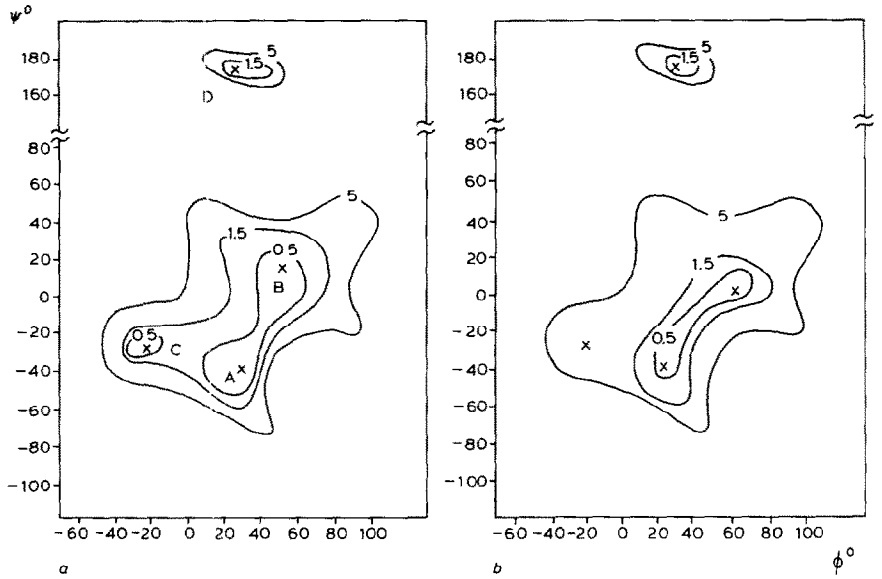

Fig. 2. Conformational maps (ϕ,ψ) of methyl β-cellobioside calculated by taking into account the non-bonded interaction functions of Scott and Scheraga[7]: (*a*) disregarding hydrogen bonds; (*b*) with hydrogen bonds. Relative energy values are indicated at equipotentials; x indicates the position of local minima (Table I).

TABLE I

TORSIONAL ANGLES (DEGREES), ENERGIES[a] (kcal/mol), COUPLING CONSTANTS (Hz), AND LINKAGE ROTATIONS (DEGREES) FOR THE OPTIMAL CONFORMATIONS OF METHYL β-CELLOBIOSIDE

Parameters	*Without hydrogen bonding*				*With hydrogen bondings*[b]			
	A	*B*	*C*	*D*	*A*	*B*	*C*	*D*
ϕ, ψ	30, −40	55, 20	−20, −25	30, 175	25, −35	60, 0	−20 −25	30, 175
U	−2.8	−2.6	−2.1	−2.6	−4.1	−4.1	−2.1	−2.6
J^{ϕ}	4.3	2.5	4.7	4.3	4.5	2.0	4.7	4.3
J^{ψ}	3.7	4.7	4.5	6.1	4.0	5.3	4.5	6.1
Λ	+122	−34	+197	+34	+123	+1	+197	+34
Statistical weights of conformers (%)[c]	39	38	10	13				

[a]Determined using the non-bonded functions of Scott and Scheraga[7]. [b]Potential[13] with the optimal hydrogen-bonding value of 2 kcal/mol was used. [c]Calculated from the free energies of the conformers.

but they are the structures in which intramolecular hydrogen-bonding O-5···HO-3′ may occur. Thus, in cellobiose, the intramolecular hydrogen-bonding may only contribute to stabilisation of conformers optimal for non-bonded interactions.

It is clear from a comparison of the observed value (+64°) of the linkage rotation for methyl β-cellobioside in aqueous solution[5] with those for optimal conformers in the disaccharide calculated by Rees' formula (Table I) that conformer A, which has a positive rotation ($\Lambda^{\phi,\psi}$ +122°), unlike the other low-energy conformer B ($\Lambda^{\phi,\psi}$ −34°), makes a considerable contribution to the conformational equilibrium of cellobiose.

The results obtained using different parametrisations of non-bonded interactions are now considered. Fig. 3 shows the dependence of the relative energy of the

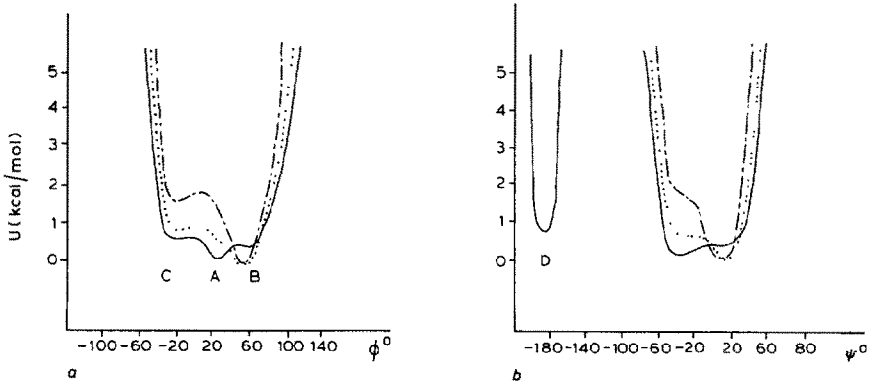

Fig. 3. Dependence of the relative energy of methyl β-cellobioside upon torsional angles ϕ (C-1–O) (*a*) and ψ (O–C-4′) (*b*), using the non-bonded interaction functions of Scott and Scheraga[7] (——), Kitaigorodsky[9] (·····), and Momany *et al.*[8] (– – –).

TABLE II

AVERAGE VALUES OF CONSTANTS J^{ϕ} AND J^{ψ} (Hz) AND LINKAGE ROTATION A (DEGREES) FOR METHYL β-CELLOBIOSIDE WITH DIFFERENT APPROXIMATIONS OF THE FORCE FIELD

Parameters	*Calculations with non-bonded interaction functions*			*Calculation by the HSEA method*[19b]	*Exptl. data*	
	Ref 7	*Ref.8*	*Ref.9*		*Methyl cellobioside*[3,5]	*Cellobiose octa-acetate*[3]
$\langle J^{\phi} \rangle$	3.9	2.7	3.2	2.2	4.2	4.2
$\langle J^{\psi} \rangle$	4.7	5.0	4.8	5.0	4.3	5.2
$\langle A \rangle$	+66	+3	+40	−4	+64	
Calculation with allowance for hydrogen bonding[a]						
$\langle J^{\phi} \rangle$	3.7	2.4	2.9	2.1		
$\langle J^{\psi} \rangle$	4.9	5.2	4.9	5.2		
$A\rangle$	+59	+11	+35	+1		

[a]An optimal hydrogen-bonding value of 2 kcal/mol was used. [b]Statistical average values are given.

disaccharide upon ϕ (C-1–O) (Fig. 3a) and ψ (O–C-4′) (Fig. 3b). For each value of ϕ among the conformers differing in angle ψ, that of lowest energy was used to plot the graphs shown in Fig. 3a. The same applies for Fig. 3b, which shows optimal energies at fixed values of ψ. It can be seen that the functions proposed by Momany *et al.*[8] and Kitaigorodsky[9] are more "rigid". In their calculations at the potential surface, a single lowest-energy minimum B (ϕ 50, ψ 10; Fig. 3a) was found, in contrast to four obtained from the calculations using the functions of Scott and Scheraga[7] (Fig. 2a).

The calculated values of $\langle A \rangle$, $\langle J^{\phi} \rangle$, and $\langle J^{\psi} \rangle$ depend on the type of parametrisation of the force field (Table II). When only non-bonded interactions (and also electrostatic interactions and torsional contributions) are taken into account, there is good agreement with the observed data, provided the functions of Scott and Scheraga are used. Thus, the calculated value of +66° for $\langle A \rangle$ fits exactly that observed (Table II). The observed and calculated values for $^3J_{C,H}$ also agree. When the functions of, for example, Momany *et al.*[8] were used, the predicted value of A was close to 0°; a substantially lower value was also obtained[3] for J^{ϕ} (2.7 Hz against 4.2 Hz).

Introduction into the calculations of the additional energy component describing the formation of hydrogen bonds has little effect on the results for methyl β-cellobioside, as can be seen from a comparison of the average values of A, J^{ϕ}, and J^{ψ} in two theoretical approximations based on identical parametrisation of non-bonded functions (*cf.* Table II, top and bottom). That the final results are practically unchanged when hydrogen bonding is taken into account is due to the fact that conformations with the lowest energy of non-bonded interactions and those with intramolecular hydrogen-bonding in cellobiose coincide (conformers A and B); their statistical contribution in the two theoretical approximations is most significant.

The theoretical conclusion that intramolecular hydrogen-bonding does not seriously change the conformational equilibrium in solutions of cellobiose is sup-

ported by the fact that the linkage rotations of methyl β-cellobioside observed in solvents of highly different polarity, namely, water and methyl sulphoxide, were[5,6] +64° and 59°, respectively. It is clear that, in methyl sulphoxide, the energy of hydrogen-bonding O-5···HO-3 must be stronger than in water.

With the parametrisation of the force field given by the HSEA approach[17,19], including Kitaigorodsky functions for non-bonded interactions and vacuum functions for the exo-anomeric effect, we failed to reproduce the observed characteristics of aqueous solutions of cellobiose (Table II, column 5). In particular, the predicted average value of the optical rotation has a negative sign (−4°), whereas the observed value is positive (+64°). The calculated value of $\langle J^{\phi} \rangle$ (2 Hz) was also considerably lower than that (4.2 Hz) observed. Moreover, on assessment of the disaccharide structure in terms of a single optimal conformer[17,18] (ϕ 60°, ψ 10°), the resulting values of the constants and linkage rotation still differ from the observed values ($\Lambda^{\phi,\psi}$ −21°, J^{ϕ} 2 Hz, J^{ψ} 5 Hz). The calculations that included a function for the exo-anomeric effect and other functions for non-bonded interactions (for example, that of Scott and Scheraga) and also for intramolecular hydrogen-bonding gave similar results (see Table II, bottom).

In this calculation model, the conformation B with an angle ϕ (C-1–O) of ~60° possesses the lowest energy because the reducing residue of cellobiose is precisely *gauche* to the linkage C-1–O-5 of the non-reducing residue; the statistical value of the other spatial conformers is negligibly small. However, such a description apparently is an oversimplification of the conformational equilibrium for cellobiose in aqueous solution. In the curve which relates J^{ϕ} and ϕ, this value of ϕ corresponds to a J^{ϕ} value of 2 Hz, whereas the observed value[3] is 4.2 Hz. Thus, a direct experimental determination of the parameter closely related to the exo-anomeric effect shows that the latter is not definitive for the understanding of disaccharide structure. At the same time, the values (4.3 and 4.7 Hz, Table I) of J^{ϕ} for conformations A and C are close to those reported[3], indicating that such conformers are present in aqueous solutions of cellobiose.

Thus, the conformational equilibrium for cellobiose in aqueous solution is best described by using the force field including only non-bonded interactions with the parametrisation of Scott and Scheraga and torsional contributions. Electrostatic interactions in non-ionised oligosaccharides are of little significance.

Such a description contains spatial forms A–D having statistical weights of 39, 38, 10, and 13%, respectively. It was possible to evaluate the contribution of each form on the basis of free energies, since the respective regions in the conformational map of cellobiose are divided by potential constraints. Cellobiose does not exist in solution in a unique rigid conformation.

It should be stressed that all of the observed characteristics of cellobiose (J^{ϕ}, J^{ψ}, and Λ) may be reproduced only when the whole potential surface of the disaccharide is taken into account. The contribution of higher energy conformers cannot be disregarded. Thus, D-type conformations (ϕ 180°) were not found in X-ray studies of oligosaccharides. However, on the basis of calculation, their contribution in sol-

ution can amount to ~13%. In fact, neglect of the D region gives a worse agreement of the calculated values of $\langle J^{\phi} \rangle$, $\langle J^{\psi} \rangle$, and $\langle \Lambda \rangle$ with the observed values.

That different spatial forms occur for cellobiose in aqueous solution is also confirmed by the fact that, in the ^{13}C-n.m.r. spectrum of cellobiose, the β-effect of glycosidation is manifested[24] in a downfield shift of -1.5 p.p.m. for the signal for C-3′ of the reducing residue on disaccharide formation. It is highly likely that this β-effect is due to "*γ-gauche*" interaction of the protons of two residues, as with glycosyl-(1→3)-galactosides[25,26]. In cellobiose, the only conformers that allow for such interactions are those of the D type, in which H-1 and H-3′ are approximately at the distance of van der Waals contacts (Fig.1). A theoretical calculation of the value of the β-effect of glycosidation in cellobiose, using the formula in ref. 26, gave $\langle \delta \rangle$ -1.1 p.p.m. Thus, for cellobiose in aqueous solution, the occurrence of a spatial form of the D type is quite likely.

Our conclusion that there is a conformational equilibrium for cellobiose in aqueous solution argues against the conclusion[5] that cellobiose in solution preserves its X-ray structure[27] with torsion angles ϕ 42° and ψ $-18°$, since such angles define an observed linkage rotation of $+64°$. The correlation of one observed value with the particular conformation cannot be regarded as proof that the molecule exists in that conformation. The correlation may be coincidental. In particular, for cellobiose, not only this structure, but also all the conformers plotted on the conformational map of the disaccharide on a straight line connecting points ϕ_1, ψ_1 60, $-30°$ and ϕ_2, ψ_2 -30,60° have a linkage rotation value equal to that observed.

Methyl β-maltoside. — The conformational map of the disaccharide (Fig. 4a)

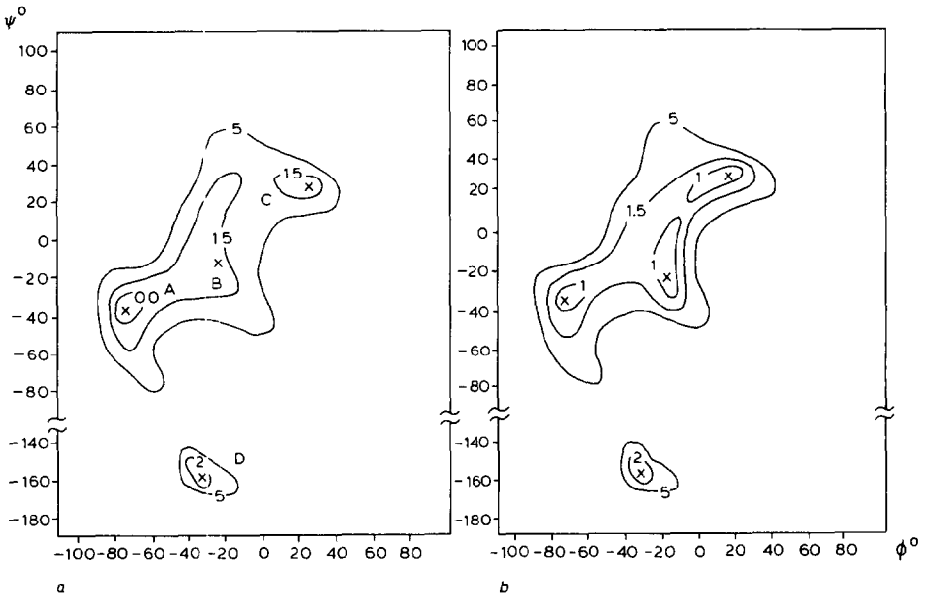

Fig. 4. Conformational maps (ϕ,ψ) of methyl β-maltoside (*cf.* legend to Fig. 2).

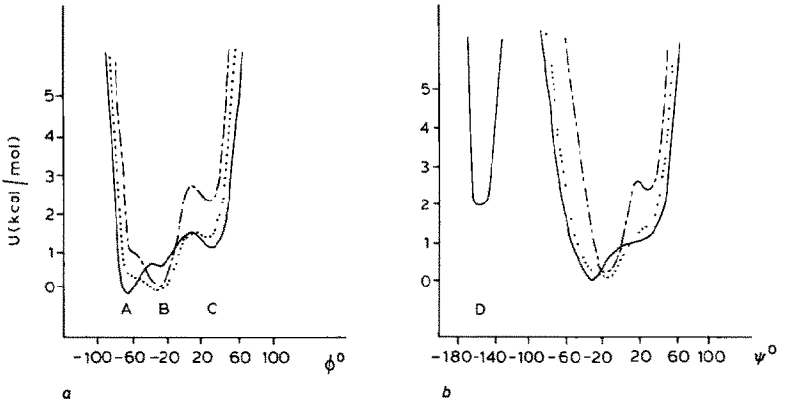

Fig. 5. Dependence of relative energy of methyl β-maltoside upon torsional angles ϕ and ψ (*cf.* legend to Fig. 3).

TABLE III

TORSIONAL ANGLES (DEGREES), ENERGIES (kcal/mol), COUPLING CONSTANTS (Hz), AND LINKAGE ROTATIONS (DEGREES) FOR THE OPTIMAL CONFORMATIONS OF METHYL β-MALTOSIDE[a]

Parameters	*Without hydrogen bonding*				*With hydrogen bonding*[b]			
	A	*B*	*C*	*D*	*A*	*B*	*C*	*D*
ϕ, ψ	−70, −35	−20, −10	30, 25	−30, −160	−70, −35	−10, −30	20, 30	−30, −160
U	−3.8	−2.9	−2.7	−2.0	−3.8	−4.3	−4.2	−2.0
J^{ϕ}	0.8	4.7	4.3	4.3	0.8	5.1	4.7	4.3
J^{ψ}	4.0	5.1	4.5	5.2	4.0	4.3	4.3	5.2
Λ	+77	−43	−225	−5	+77	−25	−205	−5
Statistical weights of conformers (%)	60	27	10	3				

[a]See footnotes to Table I.

drawn using the atom–atom potential functions of Scott and Scheraga contains four local minima (Table III), of which minimum A has the lowest energy of non-bonded interactions (ϕ −70°, ψ −35°). At the same time, when the calculations included the functions of Momany *et al.* and Kitaigorodsky, minima A and D were lacking (Fig. 5) and minimum B (ϕ −20°, ψ −10°) had the lowest energy.

The values of linkage rotations for the optimal conformers A–D are summarised in Table III. Since the observed value[6] of Λ for an aqueous solution of methyl β-maltoside is positive and equal to +46°, it follows that the contribution of conformations of the A type which, unlike others, may define a positive sign of the linkage rotation, must be substantial (Table III). Consequently, the non-bonded interaction functions proposed by Momany *et al.* and Kitaigorodsky, which give potential surfaces without minimum A (Fig. 5), give unsatisfactory results.

Calculations that took into account only non-bonded and electrostatic in-

teractions and torsional energy gave satisfactory results, *i.e.*, mean values of $\langle J^{\phi} \rangle$, $\langle J^{\psi} \rangle$, *and* $\langle \Lambda \rangle$ were obtained with the functions of Scott and Sheraga (column 2, Table IV). Only with this approximation was it possible to obtain a positive sign for the linkage rotation. Thus, the contribution of the spatial forms of the A type to the conformational equilibrium of maltose is large. Nevertheless, the calculated value of $\langle \Lambda \rangle$ is lower than that observed, suggesting that the relative energy of conformer A in Table III may be overestimated.

The calculations of J^{ϕ} using the same parametrisation were satisfactory, the calculated average value (2.8 Hz, column 2, Table IV) being very close to that (3 Hz) observed[2] for methyl β-maltoside. Conversely, a calculation of $\langle J^{\phi} \rangle$ with the functions of Momany *et al.* and Kitaigorodsky underestimates the contribution of form A and is less satisfactory, the predicated J^{ϕ} value being 4 Hz. The calculated value of $\langle J^{\psi} \rangle$ is somewhat overestimated, 4.3 Hz being the best variant (Table IV) against observed values of 2.5 Hz for methyl β-maltoside, ~3.5 Hz for maltose, and ~4 Hz for methyl β-maltoside octa-acetate[2]. However, a J^{ψ} value of <4 Hz cannot be calculated, since, for ψ in possible conformations (from −40 to +40°, Table III), the J^{ψ} values (on the curve of $^3J_{C,H}$ dependence upon ψ proposed by Perlin *et al.*[3]) are ≥4 Hz.

According to the calculations for methyl β-maltoside, disregarding and regarding intramolecular hydrogen-bonding, the conformers of lowest energy differ. It is the A structure in the former case, and the B and C structures with intramolecular hydrogen-bonding O-2···O-3 (Table III) in the latter. Therefore, the conformational equilibrium for maltose in solution, unlike, for example, that for cellobiose (see above), must be sensitive to the nature of the solvent. This finding permits an evaluation of the role of intramolecular hydrogen-bonding in disaccharides in aqueous solution.

TABLE IV

AVERAGE VALUES OF J^{ϕ} AND J^{ψ} (Hz) AND LINKAGE ROTATION Λ (DEGREES) FOR METHYL β-MALTOSIDE WITH DIFFERENT APPROXIMATIONS OF THE FORCE FIELD[a]

Parameters	*Calculations with non-bonded interaction functions*			*Calculation by the HSEA method*[19]	*Exptl. data*		
	Ref. 7	*Ref. 8*	*Ref. 9*		*Maltose*[2,6]	*Methyl β-maltoside*[2,5]	*Maltose octa-acetate*[2]
$\langle J^{\phi} \rangle$	2.8	3.9	3.8	2.4	3.5	3.0	2.0
$\langle J^{\psi} \rangle$	4.2	4.7	4.6	4.6	3.5	2.5	4.0
$\langle \Lambda \rangle$	+16	−2	−11	+13	+39	+46	
Calculation with allowance for hydrogen bonding							
$\langle J^{\phi} \rangle$	4.1	4.6	4.7	4.1			
$\langle J^{\psi} \rangle$	4.5	4.6	4.7	4.7			
$\langle \Lambda \rangle$	−50	−31	−55	−10			

[a]See footnote to Table II.

Incorporation into the force field of potential hydrogen-bonding, with optimal energy values of -1 and -2 kcal/mol, gave a considerably worse agreement of the calculated values with those observed for methyl β-maltoside in aqueous solution (Table IV, bottom). Particularly, $\langle\Lambda\rangle$ had a negative sign. In the calculation of J^{Φ} and J^{Ψ}, the error is much higher (*cf.* the data in Table IV, column 2, bottom and last columns). It follows that, in water, no additional energy stabilisation of the disaccharide conformations occurs at the expense of intramolecular hydrogen-bonding OH···O. If, under these conditions, hydrogen bonding occurred, then the contribution of conformers B and C would be large and, consequently, the disaccharide solution would have a negative linkage rotation Λ, which contradicts the fact.

With the approximation that takes hydrogen bonding into account, good agreement was obtained with the rotation of methyl β-maltoside in methyl sulphoxide[5] (Λ − 29°). From a comparison of the calculated $\langle\Lambda\rangle$ values ($-16°$ and $-50°$), obtained using optimal hydrogen-bonding energies of -1 and 2 kcal/mol and the functions of Scott and Scheraga (Table IV, bottom, column 2), with the observed values, it can be concluded that the energy of intramolecular hydrogen-bonding OH···O of oligosaccharides in solution in methyl sulphoxide is ~ -1.5 kcal/mol. The formation of intramolecular bonding O-2···HO-3′ for maltose in this solvent has been proved by proton chemical shifts in the n.m.r. spectra[28].

Thus, for maltose in aqueous solution, the enthalpy of intramolecular hydrogen bonding OH···O is zero. This is valid for cellobiose, since all the parameters are reproduced most satisfactorily when only non-bonded interactions are taken into account. In cellobiose, the statistical weight of conformers (A and B) in which such bonding can occur is highest, but the additional energy stabilisation from hydrogen bonding in aqueous solution is lacking.

The energy component associated with the exo-anomeric effect in maltose is negligible, as the results obtained were not an improvement over those obtained by neglecting the effect. A comparison of the data in Table IV (top, column 2), considered to be most satisfactory for the disaccharide in solution, with those in column 4 calculated by Lemieux's method[19] shows that, when the $\langle\Lambda\rangle$ values have a positive sign, the calculated values of $\langle J^{\Phi}\rangle$ and $\langle J^{\Psi}\rangle$ are less satisfactory.

Thus, if the exo-anomeric effect for carbohydrates in aqueous media were important, the allowance for the effect would give satisfactory results for both methyl β-maltoside and methyl β-cellobioside, which does not correspond with reality (see Tables II and IV). Therefore, in general, and consistent with the theoretical results of Tvaroška and Kozar[29], it is unnecessary to take this effect into account. Moreover, a small additional stabilisation of conformer A in maltose (~ -0.3) kcal/mol), required to obtain an average $\langle\Lambda\rangle$ value closer to that observed, may be due to hydrophobic, but not electronic, interactions in water. The possibility of hydrophobic stabilisation of "folded" conformations (type A) due to the proximity of hydrogens of carbon atoms of the two residues in maltose has been reported[6].

Thus, as for methyl β-cellobioside, the most satisfactory conformational calculations for methyl β-maltoside involves only non-bonded interactions and also torsional contributions. With such theoretical approximations, the statistical contributions of conformers A–D in methyl β-maltoside were 60, 27, 10, and 3%, respectively, *i.e.*, in aqueous solution, the A-type conformer preponderates. The contribution of conformer D, which reflects the β-effect of glycosidation due to the "*γ-gauche*" interaction of H-1 and H-3′, is negligible. The calculated[26] β-effect is zero. In fact, the ^{13}C-n.m.r. spectra of maltose showed no downfield shift of the signal for C-3′ as compared to that of D-glucose[2,5]. This conflicts with the description of the conformational equilibrium in maltose by Tvaroška[30], where the contribution of conformer D was calculated to be 30.5%. The results of the calculations of Melberg and Rasmussen for maltose[31], which gave a distribution of conformers A–D of 15, 59, 19, and 7%, respectively, are also not satisfactory. In this case, the contribution of conformations B and C that can form hydrogen bonds O-2···O-3′ is estimated to be ~80%, which is not true for aqueous solutions where, on the statistical weight of conformers, A must be the highest. Hence, the suggestion[30] that hydrogen bonding O-2···O-3′ observed in maltose crystals is not destroyed in aqueous solution is erroneous.

Thus, a comparison of the experimental data of the methyl glycosides of cellobiose and maltose with the calculated values obtained with different approximations of the force field showed that the conformational distribution in aqueous solutions could be described optimally when only the non-bonded interactions were taken into account using the parametrisation of Scott and Scheraga[7] as well as torsional contributions with a low value for the constraint of rotation[10] around the glycosidic bonds, *i.e.*, 0.9 kcal/mol. Allowance for electrostatic interactions did not affect the mean values of any of the parameters. Thus, non-bonded interaction functions in aqueous solutions of di- and oligo-saccharides are, at the same time, potentials of average force. This conclusion is supported by the calculations for methyl β-lactoside. The conformational analysis of this disaccharide permits a comparison of several calculated and observed values; not only $^{3}J_{^{13}C',H}$ but also $^{3}J_{^{13}C,^{13}C}$ defined by the torsion angles ϕ (C-1-O) and ψ (O-C-4′), namely, $^{3}J_{C\text{-}1,C\text{-}3'}$, $^{3}J_{C\text{-}1,C\text{-}5'}$, and $^{3}J_{C\text{-}2,C\text{-}4'}$, were determined for methyl β-lactoside[4]. Their observed and theoret-

TABLE V

CALCULATED AND EXPERIMENTAL VALUES OF COUPLING CONSTANTS (Hz) AND LINKAGE ROTATION (DEGREES) FOR METHYL β-LACTOSIDE

Parameters	*Calc.*	*Exptl. data*[4,5]
$\langle J^{\phi} \rangle$	3.7	3.8
$\langle J^{\psi} \rangle$	4.6	4.9
$\langle J_{C\text{-}1,C\text{-}3'} \rangle$	0.7	0
$\langle J_{C\text{-}1,C\text{-}5'} \rangle$	1.5	1.6
$\langle J_{C\text{-}2,C\text{-}4'} \rangle$	3.0	3.1
$\langle A \rangle$	+85	+86

ical values calculated with preferable parametrisation of the force field are summarised in Table V. There is good agreement with the observed values not only of the J values[3] for aqueous solutions, but also of the linkage rotation (Λ) of the glycosidic bond (Λ_{obs} +86°[5], Λ_{calc} +85°).

It should be stressed that, for disaccharides in solution, there is a complex conformational equilibrium defined by the total potential-energy surface of the molecule. There are no rigid disaccharide structures in solution. Consequently, a fit of a particular disaccharide conformation to a certain observed parameter cannot be regarded as a proof of the existence of the conformation (see ref. 32). Theoretical analysis that takes into consideration the whole conformational distribution for disaccharides gives the most satisfactory agreement with all the observed parameters.

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