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CONFORMATIONAL-ENERGY CALCULATIONS FOR OLIGOSACCHARIDES: A COMPARISON OF METHODS AND A STRATEGY OF CALCULATION*

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ABSTRACT

A theoretical conformational analysis of dimethoxymethane, 2-methoxytetrahydropyran, cellobiose, and maltose has been performed. The validity of several commonly used classical approaches to conformational energy, assuming non-bonded interactions, torsional terms, and the exo-anomeric contribution, and the MM2CARB method (a modified version of the MM2 force-field program using the Jeffrey–Taylor parameters) was tested against available experimental data or previous quantum-chemical calculations. The MM2CARB method correctly reproduces the energies and the variations in bond lengths and bond angles for conformers of dimethoxymethane and 2-methoxytetrahydropyran. Prediction of the observed conformers with simple potential functions appears to be less satisfactory. In particular, calculations that take into account non-bonded interactions and the exo-anomeric contribution based on dimethoxymethane give predicted energy differences that are 2–3 times higher than the experimental values. The general shapes of the (Φ, Ψ) potential-energy surfaces for cellobiose and maltose provided by potential-function calculations suggest the presence of several minima whose energies depend, to a great extent, on the choice of molecular geometry. The MM2CARB-calculated structures of seven cellobiose and five maltose conformers demonstrate clearly the variation of disaccharide geometry with change of conformation around the glycosidic linkage. The relative energies calculated by simple methods differ from MM2CARB energies and indicate that the simple potential-functions methods give only a qualitative estimate of oligosaccharide conformers. Based on these results, we propose a general strategy and two different approaches for the investigation of conformational properties of oligosaccharides.

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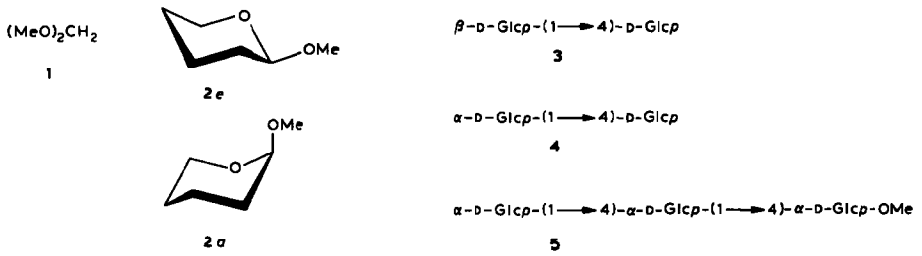
INTRODUCTION

Recognition of the relation between molecular conformations and biological function of oligosaccharides has stimulated interest in their structure and conformational properties in solution. Crystallographic studies have been very useful, as they provide information on bond lengths, bond angles, and torsion angles in the crystal lattice. However, interactions in the crystal are subject to packing constraints and to the optimization of intramolecular and intermolecular interactions. Such constraints do not apply with dilute solutions, where solute–solvent interactions are of much greater importance. Measurements of optical rotation clearly show that the equilibrium state adopted by simple oligosaccharides is very sensitive to the composition of the solvent medium[1]. High-resolution n.m.r. spectroscopy has become an important tool for studying the conformation of oligosaccharides in solution[2]. Chemical shifts, coupling constants, and relaxation rates have been used for this purpose. However, since oligosaccharides have several degrees of internal motional freedom, the observed parameters represent thermodynamically averaged values. Thus, the "average" conformation deduced from n.m.r. measurements may not have any direct physical significance, and may not correspond to any of the actual molecular conformations[3]. Furthermore, it is impossible to separate the contributions of individual conformers in an equilibrium mixture without first knowing all possible conformations included in the average, and their associated n.m.r. parameters. Conformational-energy calculations have been used to overcome such difficulties in the interpretation of experimental data that are far from trivial[4–7]. The abundance of any individual conformation is then predicted by its relative energy according to the Boltzmann principle.

The conformational energies of oligosaccharides are calculable, in principle, from either classical, "so-called" empirical, or by quantum-mechanical procedures. The latter methods and their application to carbohydrates have been reviewed elsewhere[8,9] and will not be discussed here. Alternatively, empirical energy-functions may be used. Various approximations to this type have been used successfully, particularly in conjunction with X-ray diffraction studies[10–13]. Much of the success of this approach is because van der Waals interactions have a dominant influence on the conformations of many oligosaccharides, and it is relatively easy to rank conformations in order of increasing or decreasing van der Waals repulsion. However, such a qualitative estimate of conformational energies is unsuitable for the prediction of the behaviour of oligosaccharides in solution. It is important to recognize that the energy calculated by either quantum-chemical or classical methods is evaluated for an isolated molecule. In order to apply these methods effectively to the solution behaviour of saccharides, solvation interactions must somehow be taken into account. Several different approaches have been suggested to this difficult problem (see ref. 14, for example). One of these has been applied successfully in connection with quantum-chemical methods to estimate the relative solvation energies of 2-methoxytetrahydropyran[15], glucose[16], maltose[7,17], and cello-

biose[18]. On the other hand, the reliability of a calculation of properties in solution depends to a large extent on the quality of the method used for calculating the energy of the isolated molecule. Therefore, the development of classical methods for oligosaccharides depends on both careful checking of the initial assumptions and more-extensive experimental verification of calculated energies.

In this report, the results of parallel conformational analyses by different empirical methods are presented for four test cases, namely dimethoxymethane (**1**), 2-methoxytetrahydropyran (**2**), cellobiose (**3**), and maltose (**4**). The dependence of the calculated conformational energies upon the geometrical parameters selected is also discussed. The comparison of calculated energies and geometries with available experimental data and previous quantum-chemical calculations leads us to propose a general strategy for the prediction of conformations of oligosaccharides in solution.



MODELS AND METHODS OF CALCULATION

Two model compounds, dimethoxymethane and 2-methoxytetrahydropyran, and two oligosaccharides, cellobiose and maltose, were studied. The first two were chosen because of the considerable experimental data available on their conformational equilibrium in non-polar solvents[19–22]. Additionally, conformational features of these compounds have been studied previously by various quantum-chemical methods and these results provide a frame of reference[15,23–27]. The following empirical approaches were used:

(*a*) The potential energy of conformers is calculated as a sum of terms representing the van der Waals attractions and repulsions, E_{nb}, and the torsional (E_t) contributions: $E_T = E_{nb} + E_t$ with the bond torsion around the glycosidic C–O bond being represented by a term having three-fold periodicity and a barrier, V_3 = 1.0 kcal/mol. We designate this method as PF.

(*b*) The potential energy of conformers is calculated as the sum of E_{nb} and E_{exo}, a term representing the exo-anomeric contribution[4] based on 4-31G *ab initio*-calculated energy of dimethoxymethane[25]. We used the abbreviation NBEA: E_T =

$E_{nb} + E_{exo}$. The only difference between this method and the original HSEA* method[4] is in the parametrization of the E_{NB} term, the HSEA using Kitaigorodsky's parametrization.

(*c*) The potential energy is composed of E_{nb}, E_t, with a three-fold rotational barrier, $V_3 = 2.0$ kcal/mol, and an E_{exo} contribution determined as the difference between PCILO-calculated energy-surfaces for 2-methoxytetrahydropyran and 2-ethyltetrahydropyran[28]. A designation for this method is PFOS: $E_T = E_{nb} + E_t + E_{exo}$.

The van der Waals interaction term, E_{nb}, in the foregoing methods was used in form of 6-12 Lennard–Jones with Scott–Scheraga parameters[29].

(*d*) MM2 is the force-field method[30], QCPE program no. 395.

(*e*) MM2CARB is the MM2 force-field program (QCPE no. 395) modified with the acetal-segment parameters of Jeffrey and Taylor[31].

The experimental gas-phase geometry[32] was used for dimethoxymethane. Examination of carbohydrate structures determined by X-ray methods revealed that the geometry of the acetal segment depends strongly on dihedral angles[33–35]. Alternatively, quantum-chemical calculations showed that the relative energies for conformers of **1** and **2** depend upon the geometry selected for calculation[24,25,27,36,37]. A conclusion of these studies was a suggestion[27] to build coordinates from so-called average geometrical parameters instead of coordinates provided by X-ray or neutron diffraction for individual carbohydrates. Therefore, in the case of 2-methoxytetrahydropyran (**2**), cellobiose, and maltose, we have compared several different intitial geometries with respect to their influence on conformational energies determined by each of the first three methods. These geometries, for cellobiose and maltose, corresponded to two different experimentally observed X-ray structures and, in the case of 2-methoxytetrahydropyran (**2**), were optimized values from either MM2 or MM2CARB methods.

RESULTS AND DISCUSSION

Dimethoxymethane (**1**). — Two-dimensional potential surfaces of **1**, calculated as a function of torsion angles around the internal C–O bonds, revealed seven minima which, for symmetrical reasons, correspond solely to the three conformers *gg*, *gt*, and *tt*. The *gg* conformer is doubly and the *gt* four-fold degenerate[8,9]. The notation for the individual conformers is based on the values of dihedral angles around the central C–O bonds. In this fashion, *gg* denotes both angles in an approximately *gauche* (60°, 60° or −60°, −60°) orientation, *tt* corresponds to a *trans, trans* (180°, 180°) orientation and *tg* to *trans, gauche* (180°, ±60° or ±60°, 180°) arrangement. Dimethoxymethane may be used as a model for the acetal segment (C-5–O-5–C-1–O-1–C) in α- and β-pyranosides. For the α

*Acronyms used in this article: HSEA, hard-sphere exoanomeric effect; QCPE, Quantum Chemistry, Program Exchange; PCILO, Perturbative Configuration Interaction with Localized Orbitals; other acronyms are defined in the text.

configuration, the angle (θ) is fixed at value close to 60°, for the β configuration, it corresponds to θ in the vicinity of 180°. On the basis of electron diffraction[32], dipole moment, and Kerr constant measurements[19], it is evident that *gg* conformer is the most stable. Uchida *et al.*[19] found that the energy for both *gt* and *tt* conformers is higher by 1.7 and 3.4 kcal/mol than for the *gg* conformer. A similar comparison for the relative energies of conformers of **1**, as calculated by different methods and measured experimentally, is shown in Table I. As the parameters of the MM2CARB, PFOS, and NBEA methods were determined separately for the α and β anomers, only energy differences arising from these configurations are included in Table I. These results show that the preference for a *gauche* orientation around the C–O bond is predicted by all methods except PF. This failure of the method based on non-bonded interactions and torsional terms to predict correctly the occurrence of a *gauche* conformer in the acetal segment has been demonstrated previously[26]. It has also been shown[26] that similar energy-difference results are obtained with different sets of functions and coefficients for E_{nb}. This is the reason why almost the same energy differences are calculated by NBEA and HSEA. A potential-energy analysis of the conformational surface of **1** revealed that a correct description of the rotameric population around C–O bonds in the acetal segment requires inclusion of lone-pair interactions on oxygen in the computational scheme[26,31]. These interactions are implicitly included, in both the PFOS and NBEA or HSEA schemes, in the energy terms for the exo-anomeric effect.

Table I shows that the energy differences between conformers, as calculated by the NBEA or HSEA methods, are considerably overestimated in comparison to the experimental values. Because calculations on dihydroxymethane[25] at the 6-31G level showed smaller conformational energy differences than those previously calculated with the 4-31G basis set, it is reasonable to expect that the more-sophisticated 6-31G calculations for **1** would also show results in closer corre-

TABLE I

COMPARISON OF CALCULATED RELATIVE ENERGIES ΔE_i OF STABLE CONFORMERS IN DIMETHOXYMETHANE (1) WITH EXPERIMENTAL VALUES

Method	*ΔE_i (kcal/mol)*			*Ref.*
	gg-gt	tg-tt	gg-tt	
Experiment	1.7	1.7	3.4	19
MM1 ($\varepsilon = 1$)	0.9	2.50	3.40	31
MM2 ($\varepsilon = 1$)	1.31	1.54	2.85	[a]
MM2CARB	1.11	1.01		[a]
PFOS	1.62	0.57		[a]
HSEA	2.57	5.12		4
NBEA	2.94	4.89		[a]
PF	−0.54	−0.84		[a]

[a]This work.

spondence with experimental data. The E_{exo} term in the HSEA method was first derived to mimic vacuum calculations on **1** with a 4-31G basis set. As energy differences between *gg* and *gt*, *tg*, and *tt*, and *gg* and *tt* conformers calculated on this basis are 2.57, 5.12, and 7.69 kcal/mol, respectively, the overestimated stabilization of *gauche* conformers in the HSEA and NBEA calculations is understandable.

2-Methoxytetrahydropyran (**2**). — The application of MM2 and MM2CARB methods to compound **2** has been recently analyzed in detail[37]; the MM2CARB method correctly predicts both the anomeric ratio and the geometric differences between anomers. The MM2CARB-calculated fraction of **2** having the methoxy group axial (**2***a*) is in the range of 80–85%, depending upon the value used for the dielectric constant in calculation of the electrostatic term. Experimental values[20–22] for non-polar solvents are in the 78–82% range.

Quantitative determination of the individual conformers **2***a* and **2***e* is not possible from experimental data in the literature. These values may be estimated by the PCILO quantum-chemical calculations[15,27]. The anomeric ratio *in vacuo* calculated by PCILO is 77:23. A strong solvent dependence of this ratio is also correctly described by the calculation. For example, the calculated abundance of **2***a* in chloroform is 71% (experimental values range from 71 to 78%), in methanol 65% (62–69%), in acetonitrile 67% (64–68%), in dimethyl sulfoxide 69% (74%), and in water 48% (52%). Because calculated values of the conformational ratio are in good agreement with experimental values, it is reasonable to conclude that the relative stabilities of individual conformers are also correctly predicted.

The PCILO calculations[27] revealed the existence of five minima for rotation around the C–O bond in **2**. For **2***a*, the lowest minimum appeared at 63° (*gg*) and the next (*gt*, or 152°) was ~1.48 kcal/mol higher in energy. In the region corresponding to the third staggered position, a broad maximum is found. For **2***e*, three minima occur. Here, the lowest energy is found for the tg_1 conformer ($-58°$), which is still 0.74 kcal/mol higher in energy than the *gg* conformer of **2***a*. The energy of the tg_2 (44°) and *tt* ($-160°$) conformations relative to the tg_1, are 1.36 and 1.62 kcal/mol, respectively. Similar energy differences are indicated by the MM2CARB method. Therefore, we discuss henceforth only the results for **2***a* and **2***e* as calculated by the PF, NBEA, and PFOS methods, using different but constant geometries. The starting geometries of both conformers of **2** were based upon geometrical parameters optimized by MM2CARB for the individual conformers. As the MM2 method predicts almost the same geometry for all conformers of **2**, this has been used also for comparison.

The potential energy of **2** was calculated as a function of the O–C–O–Me torsion angle (Φ), and results are illustrated graphically in Figs. 1 and 2. The mole fractions of the individual conformers in **2***a* and **2***e* were calculated from relative energies according to the Boltzmann principle. These results, together with relative energies are summarized in Tables II and III.

The general features of the energy profiles are as expected. In **2***a*, the lowest minimum occurs at about $+60°$ to $+70°$ with a wide second, but higher, minimum

TABLE II

CALCULATED RELATIVE ENERGIES ΔE_i (IN KCAL/MOL) AND MOLAR FRACTIONS X_i (IN %) FOR CONFORMERS OF 2-METHOXYTETRAHYDROPYRAN (*2a*)

Method	*Geometry*	*ΔE_i (kcal/mol)*		*X_i (%)*	
		gg	gt	gg	gt
MM2CARB	opt.	0.0	1.74	95.0	5.0
MM1CARB[a]	opt.	0.0	1.50	92.6	7.4
MM2	opt.	0.0	—	100.0	—
PFOS	*gg*	0.0	3.03	99.4	0.6
	gt	0.0	2.01	96.7	3.3
	MM2	0.0	2.44	98.4	1.6
HSEA	*gg*	0.0	4.22	99.9	0.1
	gt	0.0	3.46	99.7	0.3
	MM2	0.0	4.01	99.9	0.1
PF	*gg*	0.0	1.12	86.9	13.1
	gt	0.0	0.10	54.2	45.8
	MM2	0.0	0.91	82.3	17.7

[a]From ref. 31.

TABLE III

CALCULATED RELATIVE ENERGIES ΔE_i (IN KCAL/MOL) AND MOLAR FRACTIONS X_i (IN %) FOR CONFORMERS OF EQUATORIAL 2-METHOXYTETRAHYDROPYRAN (*2e*)

Method	*Geometry*	*ΔE_i (kcal/mol)*			*X_i (%)*		
		tg_1	tt	tg_2	tg_1	tt	tg_2
MM2CARB	opt.	0.0	1.68	1.45	87.3	5.1	7.6
MM1CARB[a]	opt.	0.0	1.50	2.50	91.4	7.3	1.3
MM2	opt.	0.0	3.11	1.92	95.8	3.7	0.5
PFOS	tg_1	0.0	1.29	3.47	89.6	10.1	0.3
	tt	0.0	0.74	2.96	77.3	22.2	0.5
	tg_2	0.0	1.14	1.21	78.4	11.4	10.2
	MM2	0.0	1.25	2.52	88.1	10.7	1.2
HSEA	tg_1	0.0	5.97	2.26	97.8	0.0	2.2
	tt	0.0	5.07	1.15	87.4	0.0	12.6
	tg_2	0.30	6.31	0.0	37.6	0.0	62.4
	MM2	0.0	5.97	1.36	90.9	0.0	9.1
PF	tg_1	0.0	0.53	1.82	68.7	28.1	3.2
	tt	0.45	0.0	1.74	30.8	65.7	3.5
	tg_2	0.30	0.68	0.0	31.4	16.5	52.1
	MM2	0.0	0.52	0.90	61.2	25.4	13.4

[a]From ref. 31.

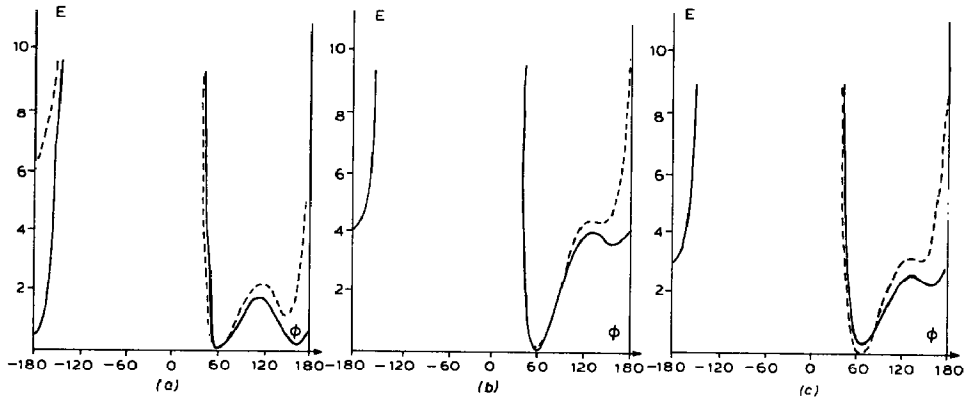


Fig. 1. Potential energy of **2***a* as a function of the torsion angle for the MM2CARB-optimized geometry for the *gg* conformer (——) and the *gt* conformer (---), respectively; (*a*) calculated by the PF method; (*b*) calculated by the NBEA method; (*c*) calculated by the PFOS method.

between +150° and +170°. The broad maximum between +40° and −160° is due to steric interactions between the methyl group and the ring and ring-substituent atoms. In **2***e* there are three minima that correspond approximately to the three staggered arrangements about the extracyclic C–O bond. The relative energies of conformers in **2***a* and **2***e*, depend on the geometrical parameters used for the calculations. In **2***a*, the calculated relative energies do not reproduce the values calculated by the PCILO or MM2CARB methods. The differences for PF are smallest, and with *gt* geometry both **2***a* and **2***e* have about the same energy. Both of the other methods yield higher differences. Of particular interest is the fact that NBEA methods predict differences of relative energy that are more than double the PCILO or MM2CARB values. The situation is similar for **2***e*. The energy

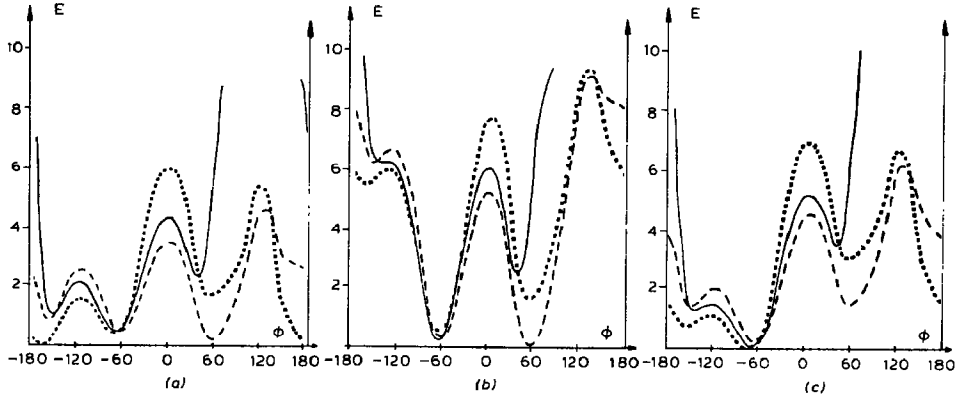


Fig. 2. Potential energy of **2***e* as a function of the torsion angle for the MM2CARB optimized geometry for the tg_1 conformer (——), tg_2 conformer (---), and *tt* conformer (· · ·) respectively; (*a*) calculated by the PF method; (*b*) calculated by the NBEA method; (*c*) calculated by the PFOS method.

differences are best reproduced by the PFOS method, although the stability of the *tt* conformer is slightly overestimated. The PF differences are strongly dependent on the assumed geometry. Thus, when tg_1 geometry is used in the starting model, the favoured conformer is predicted to be tg_1; similarly with *tt* initial geometry, the *tt* conformer is favoured, and with the tg_2 geometry the tg_2 conformer. The NBEA energy of the *tt* conformer is three times that predicted by PCILO or MM2CARB methods. The origin of this excessive destabilization of *tt* and unrealistic stabilization of *tg* conformations by the NBEA methods can be found in the derivation of E_{exo} terms. First, the *ab initio* energies (which NBEA mimic) overestimate *trans–gauche* differences by at least a factor of two. Secondly, an even more-fundamental problem arises in the application the HSEA or NBEA method to cyclic molecules. By adopting dimethoxymethane as a model for the derivation of E_{exo} terms in the HSEA method, such terms are symmetrical about 180°. However, the E_{exo} terms implicity express the interaction between the lone electron-pairs on oxygen and these interactions are symmetrical only for the conformation having the C–O bond at 180°. To avoid this bias in the HSEA E_{exo} terms, these terms could be used only for the internal torsion angles 0–180°. This, however, introduces an even more-artificial character to the HSEA method from a physical viewpoint, as the rotational potential about the C–O bond is not a continuous function of the torsion angle Φ (Fig. 3). In both **2***a* and **2***e* the discontinuities appear at 0° and 180°. Therefore, we

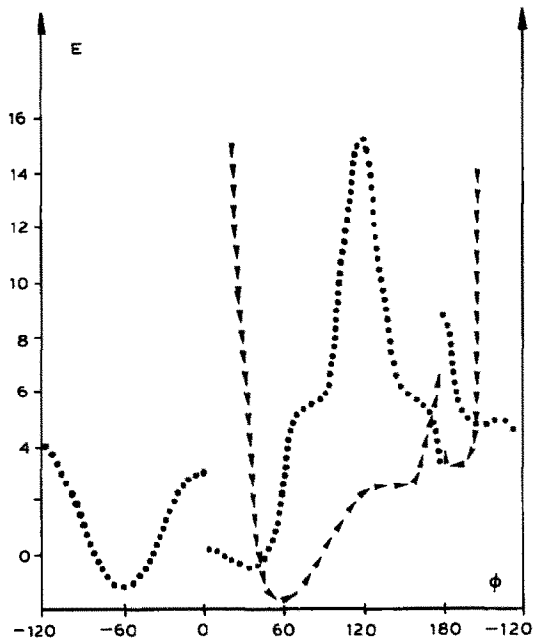


Fig. 3. Potential energy of **2***a* (——) and **2***e* (---) as a function of torsion angle Φ, calculated by the NBEA method with the exo-anomeric term defined for 0–180° intervals.

have decided to use the reported E_{exo} terms in the NBEA method for all conformations.

Cellobiose (**3**). — Two-dimensional (Φ, Ψ) energy-contour maps calculated for **3** by the PF, NBEA, and PFOS methods, using geometrical parameters from the crystal structure[38] of **3**, are given in Fig. 4, and those calculated with geometrical parameters from the crystal structure[39] of methyl β-cellobioside methanolate (**3**-Me) are in Fig. 5. The coordinates of the hydrogen atoms were determined by using a C–H bond-length of 0.11 nm and a bond vector defined by the appropriate C–C and C–O bond vectors. Maps calculated with the same fixed geometrical parameters but different algorithms have similar shapes but differ in detail. A strong influence of selected stereochemical constants (bond lengths, bond angles, and invariant torsion angles) on the (Φ, Ψ) surface is demonstrated by the different overall shapes of (Φ, Ψ) maps calculated with the same algorithm but two different sets of sterochemical constants. However, there are some similarities in the performance of the methods applied. With each geometry, the NBEA method predicts more restricted minima than the other two methods. The NBEA maps shows two steep, narrow wells, centered at $\Phi \sim -60°$ and $+50°$. All other minima have significantly higher energies. Inspection of all maps revealed the presence of eight minima whose relative energies and molar fractions are given in Table IV. These data show that relative energies of cellobiose conformers, as calculated by the three individual methods, depend considerably on the geometrical constants assumed. The most pronounced effect is observed with the NBEA method, where the choice of pyranose-ring geometry results in a markedly different predicted global minimum. Thus, when cellobiose geometry is employed, the NBEA method predicts a preference for the conformer corresponding to Φ and Ψ values observed in this crystal structure, whereas when methyl β-cellobioside ring geometry is assumed, the favoured conformer corresponds to that observed in the crystal structure of methyl β-cellobioside. With either PF or PFOS, the favoured conformer is unchanged by the assumed ring geometry, but the energy difference between two most-stable minima decreases by $\sim$0.5 kcal. Conformers corresponding to the eight lowest minima on the (Φ, Ψ) maps were used as starting points for the MM2CARB calculation.

The eight starting conformers were minimized to seven different local minima and their energies, mole fractions, and selected geometrical parameters are listed in Table V. Minimization from an area corresponding to the minimum **3**-8 leads always to the minimum **3**-2. As calculated by MM2CARB, the lowest minimum, **3**-2, appeared at ($-65°$, $-120°$); the next, **3**-1, has $\sim$0.54 kcal/mol higher energy at ($-88°$, $-175°$). The **3**-2 minium is close to the crystal-structure conformation of cellobiose ($-76°$, $-132°$), whereas the minimum **3**-1 approaches the crystal structure conformation of methyl β-cellobioside ($-91°$, $-161°$). The geometrical parameters in Table V demonstrate clearly the interaction between cellobiose ring-geometry and the conformation around glycosidic and aglycon bonds. The O-5–C-1 and C-1–O-1 bond-lengths in cellobiose conformers differ appreciably as do the

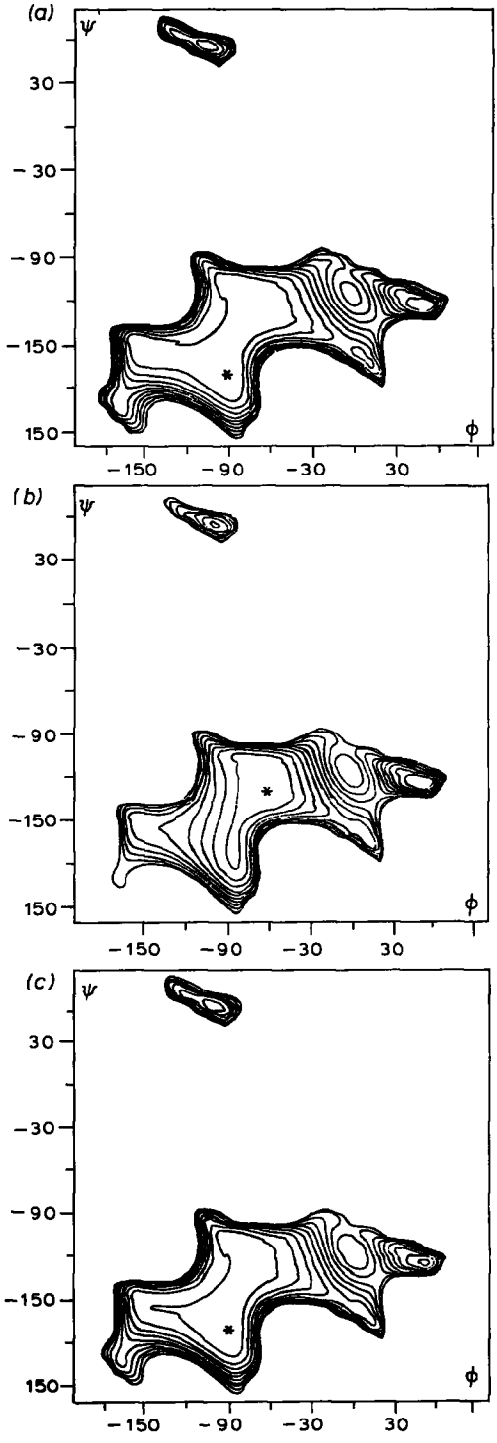


Fig. 4. Energy surface (kcal/mol) for cellobiose with geometrical parameters from the crystal structure of cellobiose as a function of torsion angles Φ and Ψ; x indicates the lowest calculated minimum; (*a*) calculated by the PF method; (*b*) calculated by the NBEA method; (*c*) calculated by the PFOS method.

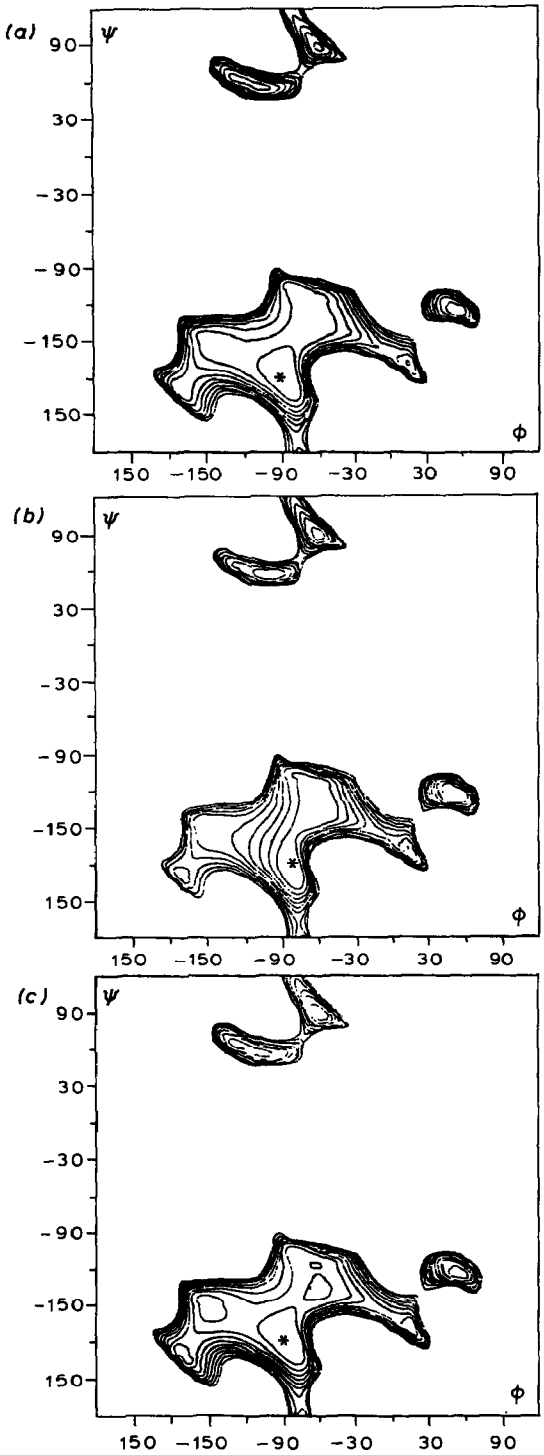


Fig. 5. Energy surface (kcal/mol) for cellobiose (3) with geometrical parameters from the crystal structure of methyl β-cellobioside, as a function of torsion angles Φ and Ψ; x indicates the lowest calculated minimum; (*a*) calculated by the PF method; (*b*) calculated by the NBEA method; (*c*) calculated by the PFOS method.

TABLE IV

RELATIVE ENERGIES ΔE_i (IN KCAL/MOL) AND MOLAR FRACTIONS X_i (IN %) FOR CELLOBIOSE CONFORMERS CALCULATED BY PF, HSEA, AND PFOS METHODS WITH GEOMETRICAL PARAMETERS CORRESPONDING TO THE CRYSTAL STRUCTURES OF CELLOBIOSE (3) AND METHYL β-CELLOBIOSIDE (3-Me)

Conformer	Φ	Ψ	ΔE_i						X_i					
			PF		*NBEA*		*PFOS*		*PF*		*NBEA*		*PFOS*	
			3	3-Me	3	3-Me	3	3-Me	3	3-Me	3	3-Me	3	3-Me
3-1	−90	−170	0.0	0.0	1.0	0.0	0.0	0.0	41.5	52.8	10.8	38.3	73.7	79.4
3-2	−60	−130	0.39	0.81	0.0	0.03	0.64	1.19	21.5	13.4	58.4	36.4	25.0	10.6
3-3	50	−120	0.65	0.96	0.38	0.29	2.57	2.88	13.9	10.4	30.8	23.5	1.0	0.6
3-4	−90	60	3.30	1.78	4.39	3.69	3.35	1.82	0.2	2.6	0.0	0.1	0.3	3.7
3-5	−150	−150	0.36	0.67	—	—	—	1.63	22.6	0.7	—	—	—	5.1
3-6	−170	−190	4.41	1.82	—	6.75	6.01	3.43	0.0	1.8	—	0.0	0.0	0.2
3-7	−50	90	—	2.57	—	1.90	—	4.64	—	2.6	—	1.6	—	−0.4
3-8	10	−160	2.94	2.96	4.30	3.31	4.60	4.64	0.3	2.7	0.0	0.1	0.0	0.0

C-5–O-5–C-1 and C-1–O-1–C-4 bond-angles. The C-1–O-1 bonds are shorter (0.1369–0.1375 nm) than the standard value of 0.143 nm and exhibit variations with (Φ, Ψ). At the same time, the O-5–C-1–O-1 bond-angle varies from 100.4 to 109.3°. Dipole moments of the individual conformers differ and are in the range 5.6–8.4 D. The variation of internal geometry as a function of the (Φ, Ψ) angles is consistent with statistical analysis of carbohydrate crystal structures[33–35]. This result also supports our suggestion, based on results[27], with **2** concerning paramount importance of structure optimization, including bond lengths and angles, in calculations of energies of carbohydrates. The comparison of relative energies in Tables IV and V shows that none of the simple empirical methods is able to predict as MM2CARB does, the same conformer stabilities for cellobiose.

Maltose (**4**). — The molecular geometrical parameters used for **4** were based on crystal structures of α-maltose[40] (**4**α) and methyl α-maltotrioside[41] (**5**). The calculated two-dimensional (Φ, Ψ) energy-contour maps are given in Figs. 6 and 7. The maps calculated by different methods but with the same geometry show a similar overall shape, but differ in the implied stabilities of conformers. However, as with cellobiose, greater differences can be observed between maps calculated by the same method but using different geometrical constants. Table VI lists the seven lowest minima found on these maps and demonstrates the significant influence of geometry upon the relative stability of conformers. The change in molecular geometry from parameters based on the crystal structure of **5** to those based on the crystal structure of **4**α generates a 1.0–2.0 kcal decrease in the relative energies of the conformers and, in the case of PFOS method, also changes the choice of favoured conformer. The influence of the geometrical parameters on the conformational preference calculated by the HSEA method for maltose has already been observed[42].

For the MM2CARB calculation, we have followed the same procedure as used with cellobiose. The seven starting conformers were minimized to five different local minima whose important characteristics are summarized in Table VII. The minimization starting from the area corresponding to the initial minimum **4**-6 leads always to the **4**-2 minimum, and that from the area corresponding to **4**-7 leads to the minimum **4**-3. The lowest minimum is the **4**-3 conformer at (88°, 77°) and the next, **4**-1, is ~0.34 kcal/mol higher energy. The **4**-2 conformer, which is closest to the crystal-structure conformation (80–150°) of **5**, lies at (88°, −147°) and has almost the same energy as the **4**-3 conformer. However none of the conformers is close to the crystal-structure conformation of **4**α (116°, −118°). Table VII also illustrates the variations of geometrical parameters as a function of conformation around the glycosidic linkage. A comparison of relative energies in Tables VI and VII shows that, as with cellobiose, the stabilities of conformers of maltose estimated by MM2CARB and by simple empirical potential methods differ significantly. In maltose, the variation in bond lengths and bond angles within the acetal segment associated with the change of conformation is smaller than in the case of cellobiose. Similarly, the difference

TABLE V

MM2CARB CALCULATED ENERGIES ΔE_i (KCAL/MOL), MOLAR FRACTIONS X_i (IN %), DIPOLE MOMENTS μ_i (IN D), AND SELECTED GEOMETRICAL PARAMETERS (BOND LENGTHS IN NM BAND ANGLES IN DEG MIN) FOR CONFORMERS OF CELLOBIOSE (3)

	3-*1*	3-*2*	3-*3*	3-*4*	3-*5*	3-*6*	3-*7*
ΔE_i	24.8226	24.2791	25.0465	25.0610	27.7496	29.2635	26.4290
X_i	20.28	50.77	13.89	13.56	0.14	0.01	1.35
Φ	−87.7	−65.0	50.7	−74.5	−148.2	−172.8	−46.6
Ψ	−175.0	−120.4	−116.6	55.2	−144.9	−176.0	83.1
Φ^H	31.8	52.9	164.2	37.7	−36.3	−63.0	70.8
Ψ^H	−59.9	1.1	4.6	173.0	−26.2	−60.6	−163.8
ω_1	61.2	63.5	62.0	61.6	64.4	65.9	61.9
ω_2	65.0	−67.3	62.5	62.3	66.9	−66.7	66.3
C-5–O-5	0.1416	0.1414	0.1414	0.1414	0.1415	0.1414	0.1412
O-5–C-1	0.1406	0.1404	0.1405	0.1403	0.1408	0.1412	0.1400
C-1–O-1	0.1369	0.1370	0.1375	0.1372	0.1373	0.1372	0.1371
O-1–C-4′	0.1402	0.1402	0.1401	0.1404	0.1401	0.1401	0.1406
C-5–O-5–C-1	113.2	113.2	113.6	113.5	113.6	114.4	113.6
O-5–C-1–O-1	107.5	106.5	109.0	109.3	103.2	100.4	108.8
C-1–O-1–C-4′	116.8	114.0	115.2	117.8	115.6	119.4	116.8
H-1–H-4′	0.2271	0.2462	0.3435	0.3572	0.2305	0.3113	0.3516
μ_i	8.4	6.50	6.2	7.50	7.9	6.1	5.6

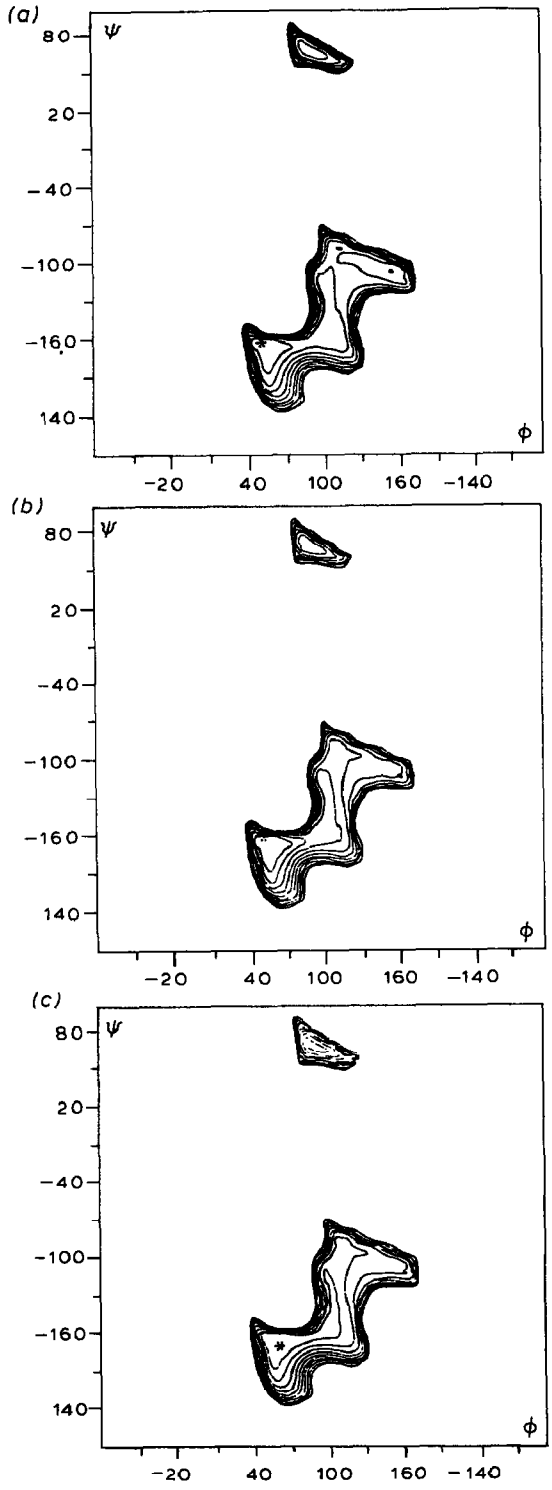


Fig. 6. Energy surace (kcal/mol) for maltose (**4**) with geometrical parameters from the crystal structure of α-maltose as a function of torsion angles Φ and Ψ; x indicates the lowest calculated minimum; (*a*) calculated by the PF method; (*b*) calculated by the NBEA method; (*c*) calculated by the PFOS method.

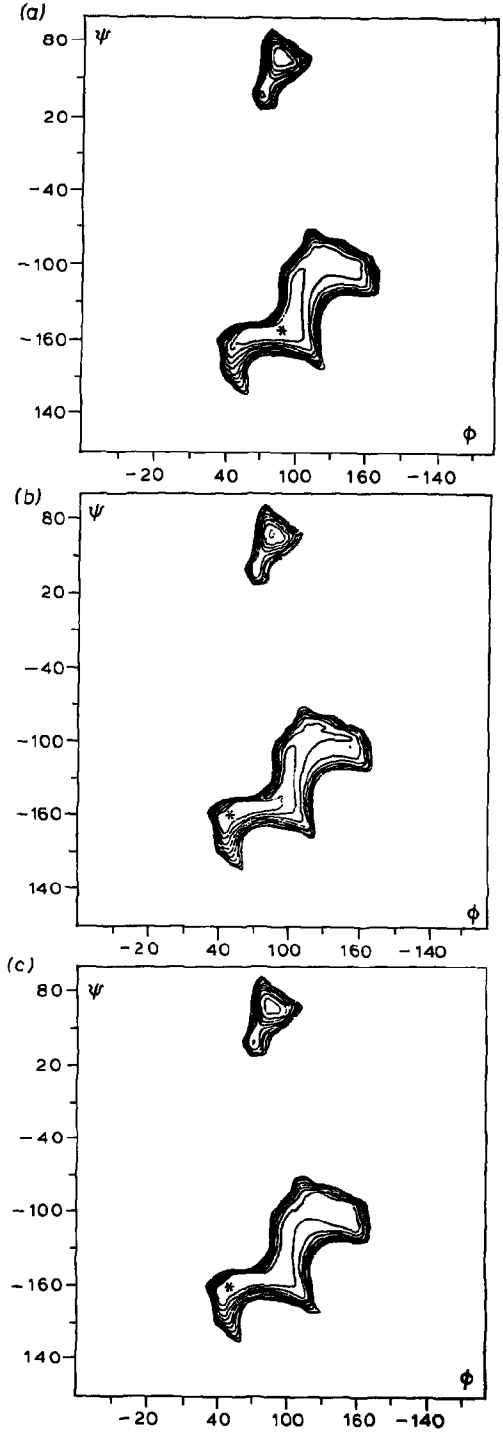


Fig. 7. Energy surface (kcal/mol) for maltose with geometrical parameters from the crystal structure of methyl α-maltotrioside as a function of torsion angles Φ and Ψ; x indicates the lowest calculated minimum; (*a*) calculated by the PF method; (*b*) calculated by the NBEA method; (*c*) calculated by the PFOS method.

TABLE VI

RELATIVE ENERGIES ΔE_i (IN KCAL/MOL) AND MOLAR FRACTIONS X_i (IN %) FOR MALTOSE CONFORMERS CALCULATED BY PF, NBEA, AND PFOS METHODS WITH GEOMETRICAL PARAMETERS CORRESPONDING TO THE CRYSTAL STRUCTURES OF α-MALTOSE (**4α**) AND MALTOTRIOSIDE (**5**)

Conformer	*Φ*	*Ψ*	ΔE_i						X_i					
			PF		*NBEA*		*PFOS*		*PF*		*NBEA*		*PFOS*	
			4α	**5**	**4α**	**5**	**4α**	**5**	**4α**	**5**	**4α**	**5**	**4α**	**5**
4-1	60	−160	0.0	0.0	0.0	0.0	0.69	0.0	31.8	82.1	67.8	96.2	14.6	64.9
4-2	90	−150	0.21	1.42	0.72	2.21	0.0	0.74	22.3	7.5	20.1	2.3	46.8	18.6
4-3	90	70	0.72	2.78	1.30	3.25	0.51	1.92	9.4	0.8	7.6	0.3	19.8	2.5
4-4	140	−100	0.12	1.77	3.18	4.82	1.71	2.79	26.0	4.1	0.3	0.0	2.6	0.6
4-5	70	40	4.10	—	4.13	—	4.05	—	0.1	—	0.1	—	0.1	—
4-6	100	−130	0.66	1.63	1.65	2.63	0.63	0.36	10.4	5.2	4.1	1.2	16.1	12.9
4-7	110	60	—	3.28	—	—	—	2.98	—	0.3	—	—	—	0.5

between the C-1–O-5 and C-1–O-1 bond lengths is smaller for the α-linked dimer. The C-1–O-1 bond lengths for maltose are longer than those in conformers of cellobiose. Likewise, the O-5–C-1–O-1 bond angles in maltose are much larger than those in conformers of cellobiose and show a significant variation (109–115°) with Φ and Ψ. All of this is consistent with a rationalization based upon dihedral-angle-dependent delocalization interactions of lone pairs on oxygen[36]. The bond-length and bond-angle variations noted here are in very good agreement with experimental data[33–35].

Differences between experimental geometrical parameters for cellobiose and maltose and the calculated ones are rather small. The maximum variations occur for the O-5–C-5 and O-1–C-4′ bond lengths, both of which are smaller than experimental ones. This is may be due to the fact that the Jeffrey–Taylor parameters were defined for the MM1 force field[31] and not for the MM2 force fields, and suggests that refinement of the original Jeffrey–Taylor parameters for the MM2 force field will yield further improvement in the agreement between experimental and theoretical values.

Strategy for conformational analysis of oligosaccharides. — The foregoing results clearly demonstrate that several conformers exist in complex equilibrium on the conformational surface of oligosaccharides. The calculated energies of **1** and **2**, the geometries of cellobiose and maltose, and previous results[37] for **2** suggest that the MM2CARB method can be applied with confidence for calculating both the relative energy and the geometry of oligosaccharide conformers.

However, the shape of the conformational surface and relative energies

TABLE VII

MM2CARB-CALCULATED ENERGIES ΔE_i (IN KCAL/MOL), MOLE FRACTIONS X_i (IN %), DIPOLE MOMENTS μ_i (IN D), AND SELECTED GEOMETRICAL PARAMETERS (BOND LENGTHS IN NM AND BOND ANGLES IN DEGREES) FOR MALTOSE CONFORMERS

	4-1	*4-2*	*4-3*	*4-4*	*4-5*
ΔE_i	21.2721	20.9561	20.9372	24.0845	23.9685
X_i	22.3	38.0	39.3	0.2	0.2
Φ	60.9	87.9	87.9	139.7	70.2
Ψ	−159.4	−146.7	77.0	−98.8	49.4
Φ^H	−57.5	−29.5	−30.3	26.1	−49.1
Ψ^H	−42.5	−29.0	−169.6	24.5	164.3
ω_1	64.9	64.2	62.1	62.5	64.3
ω_2	59.4	59.8	64.5	57.8	68.4
C-5–O-5	0.1424	0.1426	0.1427	0.1424	0.1425
O-5–C-1	0.1401	0.1402	0.1404	0.1404	0.1401
C-1–O-1	0.1388	0.1391	0.1388	0.1389	0.1389
O-1–C-4′	0.1404	0.1404	0.1407	0.1401	0.1409
C-5–O-5–C-1	113.5	113.3	113.8	113.3	114.5
O-5–C-1–O-1	113.8	111.9	112.1	109.4	115.4
C-1–O-1–C-4′	115.8	114.1	116.3	116.4	117.5
H-1–H-4′	0.2745	0.2225	0.3528	0.2210	0.3488
μ_i	6.2	6.5	6.8	6.8	5.9

calculated by the PF, NBEA, and PFOS methods depend to a great extent on the choice of molecular geometry. Investigation of the relative energies of conformers of cellobiose and maltose showed[6] that the HSEA calculations, with original Kitaigorodsky functions for non-bonded interactions or with other functions such as those proposed by Scott and Scheraga, gave similar results. These findings, together with ours on dimethoxymethane[26], suggest that the conclusions based on NBEA calculations may be extended to HSEA calculations. Because of excessive stabilization of *gauche* conformations in the NBEA method, the calculated flexibility of oligosaccharides is highly restricted. The form of the exo-anomeric contribution in this method virtually guarantees that very deep minima will be found in the vicinity of $\Phi = 60°$ and $-60°$, whereas other conformers are at much higher energies and some of them frequently cannot be found even in the allowed domains. Because of the E_{exo} term, the application of the HSEA method to oligosaccharides has been already questioned by Kochetkov *et al.*[6]. Moreover, from a physical viewpoint, there are some inconsistencies in the HSEA method, as already discussed here. Therefore, this method should be avoided for the calculation of conformational properties of oligosaccharides.

Although the other two methods, PF and PFOS, do not suffer from the same limitations and biases of the HSEA or NBEA approach, the relative energies calculated by these methods also differ from those predicted by MM2CARB; these methods can be used only for qualitative description of the conformational energy-surface for oligosaccharides. One additional term which may have significant influence on oligosaccharide shapes is the hydrogen-bond energy contribution. This term is included routinely in the PFOS energy scheme[7] and we have also introduced a hyrogen-bond energy term into the MM2CARB force field in the same way as was done[31] in MM1CARB. As this term is not used in either the PF, NBEA, or HSEA methods, we did not include its contributions in comparing the different programs. However, the contribution of hydrogen-bond energy to stabilization of oligosaccharides may be very important and should be taken into account for a quantitative estimate of relative energies.

It is important, however, to be aware of the MM2CARB characteristics if inappropriate applications and subsequent interpretations of the results are to be avoided. These characteristics involve, first, the problems concerned with the localization of minima on a very-complex energy hypersurface and, secondly, the fact that the calculated energies correspond to the situation *in vacuo*. As the oligosaccharide conformers differ in both molecular geometry and charge distribution (see Tables V and VII), it is clear that the solvation properties of these conformers will differ. The strong influence of the medium on the conformational equilibrium of **2** is an illustration of this effect[15]. Therefore, it is necessary to take solvation contributions into account when comparing experimental data from solution with calculated values, or when using theoretical models for interpretating experimental data. Neglect of this fact biases the procedure and the results may be very misleading.

All of the foregoing results indicate two possible approaches or strategies in the investigation of conformational properties of oligosaccharides when used in a simulation of oligosaccharide behaviour in solution. First, the two-dimensional (Φ, Ψ) energy-surface is calculated by the MM2CARB method, optimizing the geometry for each (Φ, Ψ) conformer. On this (Φ, Ψ) map, corresponding to the isolated molecule, it is necessary to superimpose the solvation-energy maps for each solvent to give different (Φ, Ψ) maps for any given solvent. Next, by relating the relative energies for each conformer to the Boltzmann principle, the average characteristics of a given oligosaccharide may be estimated and compared with experimental values. However, the foregoing approach requires considerable effort and computer time, and is probably feasible only for simple disaccharides. Therefore, as an alternative, we propose the following simpler methodology. In the first step, a two-dimensional (Φ, Ψ) energy-surface is calculated by either the PFOS or the PF method with a molecular geometry generated from average coordinates of Scott and Arnott[43] or Sheldrick and Akrigg[44]. This map is used for defining the regions of local minima. In the next step, the local minima are used as starting points for optimization of geometry and for precise specification of minima on the (Φ, Ψ) surface. At this point, the contribution of solvation energy is superposed onto the MM2CARB energy for each individual minimum. The average properties are calculated in this case only from minima determined on the (Φ, Ψ) surface and are compared with experimental data. Although this approach is usually sufficient, oligosaccharides may exist in which, on the solvent-specific (Φ, Ψ) surface, there appear one or more minima that are not present on the (Φ, Ψ) surface of the isolated molecule. This situation will not occur in the first approach.

Theoretical conformational analysis using the foregoing approaches takes into account the whole conformational distribution of oligosaccharides in solution. The comparison of calculated results with experimentally observed parameters will permit better understanding of the solution behaviour of oligosaccharides on the one hand, and to improved methods of theoretical conformational analysis on the other.

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REFERENCES

1 D. A. Rees and D. Thom, *J. Chem. Soc. Perkin Trans. 2,* (1977) 191–201.
2 K. Bock, *Pure Appl. Chem.*, 55 (1983) 605–622.
3 O. Jardetzky, *Biochem. Biophys. Acta,* 621 (1980) 227–232.
4 H. Thøgersen, R. U. Lemieux, K. Bock, and B. Mayer, *Can. J. Chem.*, 60 (1982) 44–57.
5 J. R. Brisson and J. P. Carver, *Biochemistry,* 22 (1982) 1362–1368.
6 G. M. Lipkind, V. E. Verousky, and N. K. Kochetkov, *Carbohydr. Res.*, 133 (1984) 1–13.
7 S. Pérez, F. Taravel, and C. Vergelati, *Nouv. J. Chim.*, (1985) 561–564.

8 I. TVAROŠKA AND T. BLEHA, *Chem. Zvesti,* 39 (1985) 805–849.
9 I. TVAROŠKA in G. NARAY-SZABO (Ed.), *Theoretical Chemistry of Biological Systems,* Elsevier, Amsterdam, 1986, 283–344.
10 P. R. SUNDARARAJAN AND V. S. R. RAO, *Tetrahedron,* 24 (1968) 289–295.
11 D. A. REES, *J. Chem. Soc., Sect. B,* (1969) 217–226.
12 P. ZUGENMAIER AND A. SARKO, *Biopolymers,* 15 (1976) 2121–2136.
13 I. TVAROŠKA, S. PÉREZ, AND R. H. MARCHESSAULT, *Carbohydr. Res.,* 61 (1978) 97–106.
14 B. PULLMANN (Ed.), *Environmental Effects on Molecular Structure and Properties, Jerusalem Symp. Quantum Chem. Biochem.,* 8 (1976), Reidel, Dordrecht, Holland.
15 I. TVAROŠKA AND T. KOZAR, *J. Am. Chem. Soc.,* 102 (1980) 6929–6936.
16 I. TVAROŠKA AND T. KOZAR, submitted for publication.
17 I. TVAROŠKA, *Biopolymers,* 21 (1982) 1887–1982.
18 I. TVAROŠKA, *Biopolymers,* 23 (1984) 1951–1960.
19 T. UCHIDA, Y. KURITA, AND M. KUBO, *J. Polym. Sci.,* 29 (1956) 365–373.
20 A. J. HOOG, H. R. BUYS, C. ALTONA, AND E. HAVINGA, *Tetrahedron,* 25 (1969) 3365–3375.
21 R. U. LEMIEUX, A. A. PAVIA, A. A. MARTIN, AND K. A. WATANABE, *Can. J. Chem.,* 47 (1969) 4427–4439.
22 C. B. ANDERSON AND D. T. SEPP, *Tetrahedron,* 24 (1968) 1707–1716.
23 I. TVAROŠKA AND T. BLEHA, *J. Mol. Struct.,* 24 (1975) 249–259.
24 D. G. GORENSTEIN AND D. KAR, *J. Am. Chem. Soc.,* 99 (1977) 672–677.
25 G. A. JEFFREY, J. A. POPLE, J. S. BINKLEY, AND S. VISHVESHWARA, *J. Am. Chem. Soc.,* 100 (1978) 373–379.
26 I. TVAROŠKA AND T. BLEHA, *Collect. Czech. Chem. Commun.,* 43 (1978) 922–931.
27 I. TVAROŠKA AND T. KOZAR, *Carbohydr. Res.,* 90 (1981) 173–185.
28 I. TVAROŠKA, *Carbohydr. Res.,* 125 (1984) 155–160.
29 R. A. SCOTT AND H. A. SCHERAGA, *J. Chem. Phys.,* 42 (1965) 2209–2215.
30 U. BURKERT AND N. L. ALLINGER, *ACS Monograph,* 117 (1982).
31 G. A. JEFFREY AND R. TAYLOR, *J. Comput. Chem.,* 1 (1980) 99–109.
32 E. E. ASTRUP, *Acta Chem. Scand.,* 25 (1971) 1494–1495.
33 I. TVAROŠKA AND T. KOZAR, *Chem. Zvesti,* 35 (1981) 425–440.
34 B. FUCHS, L. SCHLEIFER, AND E. TARTAKOVSKY, *Nouv. J. Chim.,* 8 (1984) 275–278.
35 G. A. JEFFREY, J. A. POPLE, AND L. RADOM, *Carbohydr. Res.,* 25 (1972) 117–131.
36 I. TVAROŠKA AND T. BLEHA, *Can. J. Chem.,* 57 (1977) 424–435.
37 T. KOZAR, A. SARKO, AND I. TVAROŠKA, submitted for publication.
38 S. S. C. CHU AND G. A. JEFFREY, *Acta Crystallogr., Sect. B,* 24 (1968) 830–838.
39 J. T. HAM, D. G. WILLIAMS, *Acta Crystallogr., Sect. B,* 26 (1970) 1373–1379.
40 F. TAKUSAGAWA AND R. A. JACOBSON, *Acta Crystallogr., Sect. B,* 34 (1978) 213–218.
41 W. PANGBORN, D. LANGS, AND S. PÉREZ, *Int. J. Biol. Macromol.,* 7 (1985) 363–369.
42 R. U. LEMIEUX AND K. BOCK, *Arch. Biochem. Biophys.,* 221 (1983) 125–134.
43 S. ARNOTT AND W. E. SCOTT, *J. Chem. Soc. Perkin Trans. 2,* (1972) 324–335.
44 B. SHELDRICK AND D. AKRIGG, *Acta Crystallogr., Sect. B,* 36 (1980) 1616–1621.