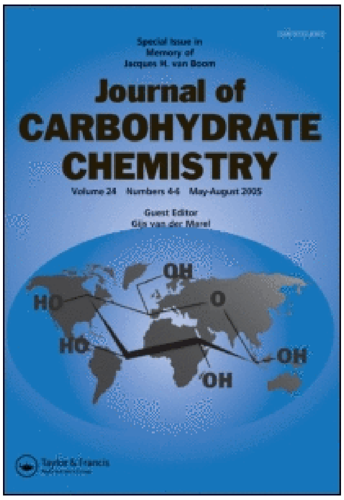


Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Use of a General Purpose Force-Field (MM2) for the Conformational Analysis of the Disaccharide α-D-Galactopyranosyl-(1 ⟶ 3)-β-D-Galactopyranose

Carlos A. Stortz[a]; Alberto S. Cerezo[a]
[a] Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Univ. Buenos Aires, Ciudad Universitaria, Buenos Aires, Argentina

To cite this Article Stortz, Carlos A. and Cerezo, Alberto S.(1994) 'Use of a General Purpose Force-Field (MM2) for the Conformational Analysis of the Disaccharide α-D-Galactopyranosyl-(1 ⟶ 3)-β-D-Galactopyranose', Journal of Carbohydrate Chemistry, 13: 2, 235 — 247

To link to this Article: DOI: 10.1080/07328309408009190

URL: http://dx.doi.org/10.1080/07328309408009190

J. CARBOHYDRATE CHEMISTRY, 13(2), 235-247 (1994)

USE OF A GENERAL PURPOSE FORCE-FIELD (MM2) FOR THE CONFORMATIONAL ANALYSIS OF THE DISACCHARIDE α-D-GALACTOPYRANOSYL-(1→3)-β-D-GALACTOPYRANOSE

Carlos A. Stortz and Alberto S. Cerezo

Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales,
Univ.Buenos Aires, Ciudad Universitaria, 1428 Buenos Aires, Argentina.

Received December 30, 1992 - Final Form September 21, 1993

ABSTRACT

Conformational analysis for disaccharide α-D-Gal*p*-(1→3)-β-D-Gal*p* was carried out by molecular mechanics calculations with the general purpose force-field MM2. The adiabatic potential energy surfaces were obtained using a total force-field, and by substracting the effects of hydrogen bonding and electrostatic forces. The effect of modifying the dielectric constant was also studied. A complex conformational equilibrium was shown. The positions and energies of the minima were also calculated with the force field MM2CARB. Linkage rotations (Λ), NOE values and interatomic distances calculated for the preferred conformations were compared with those from experimental data; it is concluded that in aqueous solutions hydrogen bonding should be discarded, and that electrostatic interactions are of little significance.

INTRODUCTION

The knowledge of conformational states of oligo- and polysaccharides is essential to evaluate their biological and physical functions. Molecular mechanics use energy functions with empirical parameters to estimate the variations of molecular energy with the position of the component atoms. A careful selection of the energy components gives rise to a useful approximation to the system.[1]

There are controversies about the kind of force-field which should be applied to carbohydrates. Lemieux and coworkers[2,3] have recommended the use of the HSEA (hard-sphere, *exo*-anomeric) method, in which the atoms are taken as rigid spheres and explicit allowance for *exo*-anomeric effect is made. Hydrogen bonding is sometimes considered,[4] even in aqueous solution, though other authors[5] disregard it. These and other authors[5,6] also disregard the *exo*-anomeric effect (at least in water) but the general trend[7,8] is to take it into account.

In this paper, a general purpose force-field (Allinger's MM2[9]) is used to investigate the conformation of the disaccharide α-D-Gal*p*-(1→3)-β-D-Gal*p*, which is not only the repeating unit of red seaweed galactans,[10] but also the antigenic determinant of blood group B.[11] Calculations were carried out using different force-field functions (as supressing hydrogen-bonding and/or electrostatic forces) and results compared with experimental data. The same data was also calculated using the force-field MM2CARB,[8] which uses a different set of constants for the atoms of the anomeric center, to allow for its associated stereo-electronic effects.

PROCEDURE

Force-field. In order to calculate the conformations of a disaccharide, it is possible to use two principles: analysis with rigid residues, where the angles and bond lengths of each of the component monosaccharides are not allowed to change, and analysis with flexible residues, which allows adjustment of all angles and bonds ("relax") to other changes in the molecule.[1] This study is based on the second approach. The program PCModel (Serena Software), which modified slightly Allinger MM2/MMP2 parameters, was used.[9] This program does not explicitly take into account the *exo*-anomeric effect. In a different approach, the MM2CARB force-field,[8] *i.e.*, newer versions of MM2 parameterized with the data of Jeffrey and Taylor[12] was also used. The energy for a given conformation is calculated as the sum of terms corresponding to torsion, stretching, electrostatic, bending,

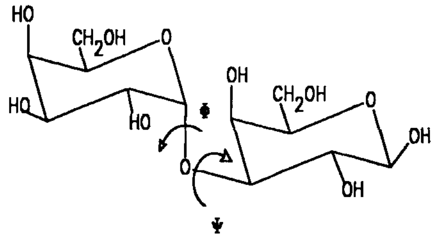


Fig.1 Disaccharide α-D-Gal*p*-(1→3)-β-D-Gal*p* and torsion angles ϕ and ψ.

van der Waals (non-bonded) and stretch-bend energies. Molecular mechanics is usually associated with obtaining an "ideal" structure, which is decided by the program by minimization of energy through automated optimization of the atomic positions.[1] Though all coordinates vary freely, the gross conformational features of a disaccharide are defined by the torsion angles around the glycosidic linkage ϕ and ψ (Fig.1), because of their higher freedom of movement. The dihedrals ϕ and ψ are defined by atoms H1'-C1'-O3-C3 and H3-C3-O3-C1' respectively, using IUPAC conventions:[13] for a dihedral A-B-C-D, the angle is 0° when looking to a Newman projection from B towards C, A is eclipsed with D; the sign is positive when D rotates clockwise respect to A.

Calculation method. The position of the minima were calculated first without any constraint; the initial conformations for each monosaccharide were those calculated as of minimum energy for α- and β-galactose, and the initial torsion angles, those resulting from observation of molecular models; the system was allowed to minimize. Different angle pairs were tried to determine and check full confluence to all possible minima. From these minima, energy was calculated (with the rigid rotor approximation) for points on a grid separated 5° in ϕ and ψ. To avoid overestimations of energy and reduce the presence of spurious geometries,[14] energy was recalculated for points distant *ca.* 20-25° in either ϕ or ψ constraining these two angles, but minimizing for variations in the rest of the atomic coordinates (*i.e.* allowing the other variables to "relax"); when in doubt, each pair of angles was used as a starting point to minimize in an iterative manner, in order to obtain the true minimum energy for each grid point (an "adiabatic" map).[14] The default dielectric constant ($\varepsilon = 1.5$) was used.

A conformational map, where the energy surface is plotted as a function of torsion angles ϕ and ψ was obtained. Only energies of grid points which were up to 5 kcal/mol above the global minimum were calculated. Though this relaxed map is a good approximation of the true energy surface, it should be considered that the map is only a small tridimensional section of the conformational surface in 202 dimensions (3 coordinates per atom or electron pair plus energy).[15]

RESULTS

The calculation was made, first by using all the energy terms, including the possibility of intramolecular hydrogen-bonding (Method **1**). Results are shown on Table 1. Figure 2a shows the conformational map obtained with these data. Three minima, each defining one region were found: region A (ϕ g^- and ψ g^+) which reaches positive values of ϕ, region B (ϕ and ψ g^-) and region C (ϕ g^- and ψ t). Minimum A is highly stabilized; besides the hydrogen-bonding common to all allowed conformations between H(O4')-O6', H(O3')-O4' and H(O4)-O6, a new inter-residual one is observed: H(O2)-O5'. Region B is separated by a potential barrier of ~ 4.1 kcal/mol, with its minimum 2.4 kcal/mol above the one from region A, but extending to a broader domain without large energy variation. Minimum B is not stabilized by inter-residual hydrogen-bonding. Region C is an "island",[16] *i.e.*, it is separated by a high energy barrier (>> 5 kcal/mol) from regions A and B. The minimum in this region has an energy similar to that of region B, but its domain is notably smaller (Figure 2a). Hydrogen-bonding between H(O2) and O2' (plus the three already mentioned) stabilizes this region.

The whole calculation was repeated, but now disregarding the possibility of intramolecular hydrogen-bonding (method **2**). Lipkind *et al.*[5] have determined that hydrogen-bonding parameters should not be included when analyzing conformations of disaccharides in aqueous solutions, though this is controversial.[17] Results are shown in Table 1 and Figure 2b. The same three regions are observed, conferring on the figure a qualitative look similar to the previous one. However, region B grew because one of the stabilizing factors of region A, the hydrogen bond between H(O2) and O5' has disappeared. Energy values for minima A and B are similar (only 0.5 kcal/mol distant), but region B is more extended. There is a considerable leveling of energy among the points of the grid: the barrier between minima A and B is lower than 2 kcal. On the other hand, the "isthmus" existing in **1** between zones A and B has greatly broadened. The island region C was reduced slightly using this method, though its relative minimum energy is similar.

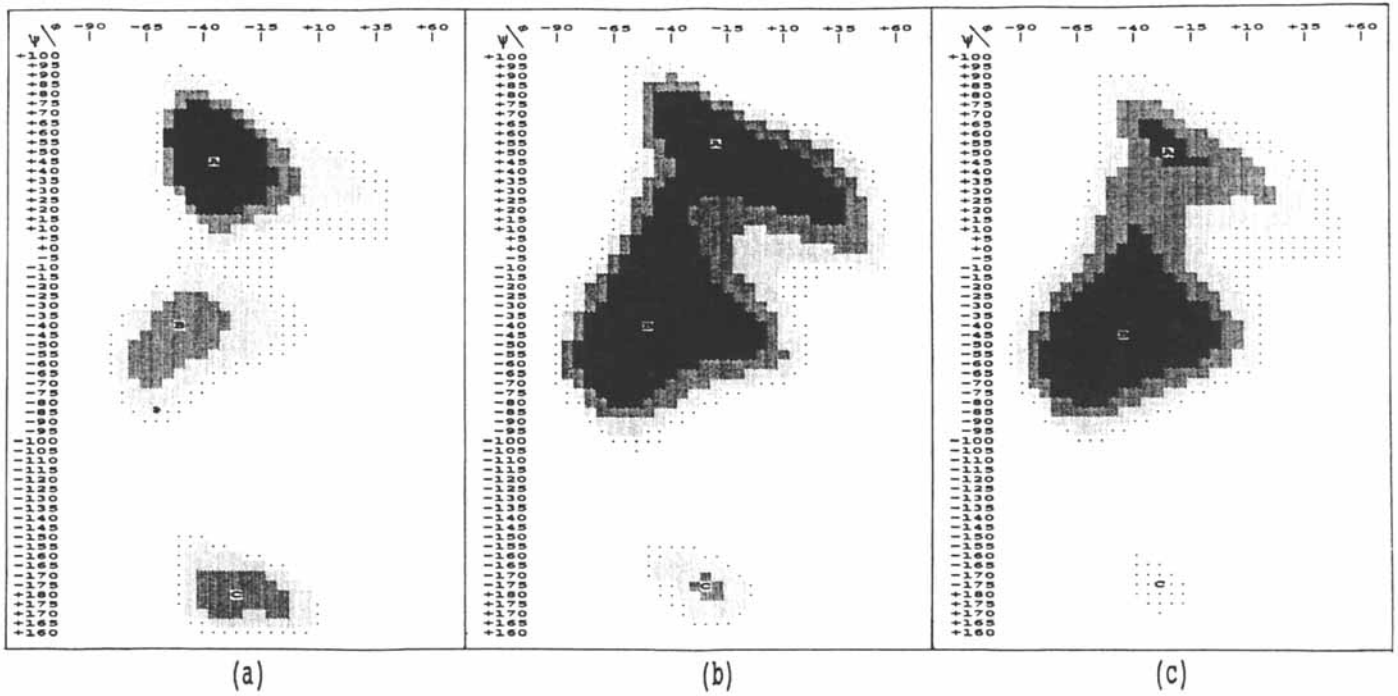
Considering that electrostatic interactions may have a meager role in a solvent with high dielectric constant like water,[5] the calculation was repeated taking out this term from the equation. Results are shown on Table 1 (method **3**) and Figure 2c. The differences between methods **1** and **2** were intensified by method **3**, *i.e.*, minimum B passed ~1.6 kcal below A, thus reducing this region. Region C has also diminished, with

TABLE 1. **Torsion angles (°), relative energies (kcal/ mol), populations (%), linkage rotations (Λ, in °), relative NOE values and H1'-H3 distances (Å) for the optimal conformations obtained for disaccharide α-Gal-(1→3)-β-Gal using MM2.**

	Method 1	Method 2	Method 3
Minimum A			
ϕ,ψ	-31, 49	-27, 58	-25, 55
E_{rel}	0.00	0.49	1.64
Population	96.9	29.8	6.3
Λ	-134	-152	-153
NOE on H3[a]	0.59	0.51	0.53
NOE on H4[a]	-0.07	-0.06	-0.07
$d_{H1'-H3}$	2.22	2.31	2.27
Minimum B			
ϕ,ψ	-55, -50	-49, -40	-48, -40
E_{rel}	2.36	0.00	0.00
Population	1.7	69.4	93.6
Λ	+85	+63	+61
NOE on H3[a]	0.09	0.19	0.19
NOE on H4[a]	0.61	0.59	0.57
$d_{H1'-H3}$	2.90	2.69	2.68
Minimum C			
ϕ,ψ	-21, -177	-24, -175	-30, -175
E_{rel}	2.45	2.66	4.11
Population	1.4	0.7	0.1
Λ	-56	-46	-35
NOE on H3[a]	0.00	0.00	0.00
NOE on H4[a]	0.04	0.04	0.03
$d_{H1'-H3}$	3.56	3.57	3.59
AVERAGE			
Λ[b]	-117 (-129)	- 12 (- 2)	+ 44 (+ 48)
NOE on H3[a]	0.58	0.29	0.21
NOE on H4[a]	-0.05	0.44	0.55
$d_{H1'-H3}$[b]	2.27 (2.25)	2.52 (2.58)	2.65 (2.65)

a. By irradiating H1', relative to the NOE on H2'.

b. Calculated by integration over the map's surface (in parenthesis the calculation over the statistical population of the minima).



(a) (b) (c)

Fig.2 Conformational maps of the disaccharide, using (a) Method 1, (b) Method 2, and (c) Method 3 within the MM2 force-field. The black colored surfaces represent areas with energies up to 1 kcal/mol the global minimum; each decreasing shade of gray is 1 kcal/mol above the previous one.

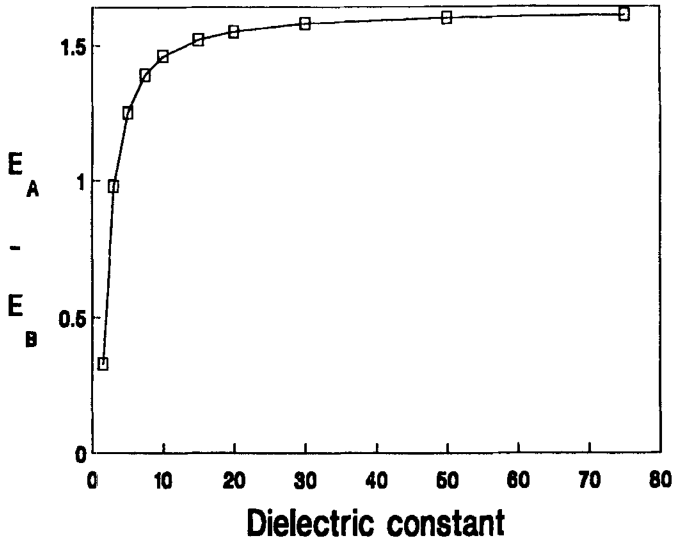


Fig. 3 Plot of difference of energy of minima A and B (in kcal/mol) *vs* dielectric constant. Fully relaxed minima were calculated in all cases.

energies which surpass the absolute minimum by more than 4 kcal/mol. The barrier between A and B is *ca.* 2.7 kcal/mol.

The minima obtained for regions A and B were recalculated using different dielectric constants. Figure 3 shows the plot of ΔE between minima A and B against dielectric constant, and Table 2 the energetic contributions to this differ-ence, and the effect of using other approaches as electrostatic dipole-dipole interactions or a distance-dependent dielectric constant.[18]

The calculations of minima were repeated, but this time using the force field MM2CARB,[8] which is especially parameterized for saccharides. However, Method **1** could not be reproduced as PCModel does not allow the use of a "special" atom as the ring oxygen as a donor for hydrogen-bonding. The results for the minima calculated with this force-field, considering and not considering electrostatic interactions (Methods **2'** and **3'**, respectively), are shown in Table 3. Dipole-dipole interactions were used for Method **2'**, as was stated,[19] giving better results. Methods **2'** and **3'**, with minimum B stabilized by 1.4-1.7 kcal/ mol respect to minimum A, gave similar results, close to those obtained using method **3** (Table 1).

TABLE 2. Differences in total energies between minima A and B (ΔE, kcal/mol) and partial contributions using different parameters for the force-field MM2 and MM2CARB.

Method	H-Bond	Electr.[a]	ε	ΔE	Contribution to ΔE from					
					Stretching	Bending	Str.-Bend	Torsion	Non-bonded	Elec.[b]
1	Yes	Q-Q	1.5	-2.36	+0.1	+0.3	0.0	+0.2	-1.0	-2.0
	Yes	D-D	1.5	-2.29	+0.1	+0.4	0.0	+0.2	-0.8	-2.3
2	No	Q-Q	1.5	+0.49	0.0	+1.1	0.0	0.0	+0.5	-1.1
	No	Q-Q	DD[c]	+0.68	0.0	+1.2	0.0	0.0	+0.4	-0.9
	No	D-D	1.5	+0.33	0.0	+1.2	0.0	0.0	+0.4	-1.3
	No	D-D	3	+0.98	+0.1	+1.2	0.0	0.0	+0.5	-0.8
	No	D-D	5	+1.25	+0.1	+1.2	0.0	0.0	+0.5	-0.5
	No	D-D	7.5	+1.39	+0.1	+1.2	0.0	0.0	+0.4	-0.3
	No	D-D	10	+1.46	+0.1	+1.2	0.0	0.0	+0.4	-0.2
	No	D-D	15	+1.52	+0.1	+1.2	0.0	0.0	+0.4	-0.2
	No	D-D	20	+1.55	+0.1	+1.1	0.0	0.0	+0.4	-0.1
	No	D-D	30	+1.58	+0.1	+1.1	0.0	+0.1	+0.4	-0.1
	No	D-D	50	+1.60	+0.1	+1.1	0.0	+0.1	+0.4	0.0
	No	D-D	75	+1.61	+0.1	+1.1	0.0	+0.1	+0.3	0.0
3	No	---	_	+1.64	+0.1	+1.1	0.0	+0.1	+0.3	0.0
2'[d]	No	D-D	1.5	+1.73	+0.1	+1.4	0.0	-0.3	0.0	+0.5
3'[d]	No	---	_	+1.43	+0.1	+1.2	0.0	-0.1	+0.2	0.0

a. Type of electrostatic force measurement: Q-Q (charge-charge) or D-D (dipole-dipole).
b. Electrostatic energy.
c. Distance dependent dielectric constant.
d. Using the MM2CARB force-field.

DISCUSSION

To check the theoretical methods with experimental data, there are different kind of values to compare with.

One of them is linkage rotation (Λ) which is related[5,20] (for α-linked disaccharides) with angles ϕ and ψ by equation: $\Lambda = -105 - 120(\sin\phi + \sin\psi)$

Though this calculation has been made[20] with ϕ and ψ values obtained from the global minimum, the best way is to weigh all possible conformations using their Boltzmann populations. Thus, an average linkage rotation can be obtained by integration along the map. Table 1 shows the result of calculating Λ by integration along the map and by considering the weights of the isolated minima. Results obtained with MM2 by the three methods are sharply different: while method **1** gives a high negative value (-117 or -129°) due almost exclusively to the presence of conformers in region A (Λ = -134°), method **2** gives a value close to zero (-2 or -12°) by averaging region A (of negative value) with B (of positive value). Method **3** gives a positive linkage rotation (+44 or +48°) close to that of the conformer representing the global minimum B(Λ = 61°); MM2CARB gives, by either method (Table 3) similar values (+34 or +40°).

Though the $[\alpha]_D$ of the β-anomer of this disaccharide is not known, the $[\alpha]_D$ of the anomeric mixture was reported.[10] The equation $\Lambda = M_{disac.} - (M_{\alpha MeGal} + M_{Gal})$, where the M are the molecular rotations, using the data reported for the monosaccharides,[20] gives a linkage rotation of +13° for the disaccharide. This result suggest that methods **2** and **3** of MM2 or MM2CARB gave a better approximation, as the Λ obtained with method **1** is too far from experimental. This agrees with Lipkind *et al.*[5] who postulated that hydrogen-bonding should not be considered in aqueous solution.

Another value to compare is the nuclear Overhauser effect (NOE), which has been determined experimentally for this disaccharide[2] by measuring the enhancement produced on the peaks corresponding to H3 and H4 in the NMR spectrum when irradiating H1'. The experimental values (relative to the NOE on H2') are 0.32 and 0.53, respectively.[2] The values obtained by calculation are shown on Tables 1 and 3. Again, method **1** gives values very far from experimental. The other four methods give values closer to experimental, but none matched for the two atoms, as methods **2** and **2'** (0.29 and 0.33, respectively) gave a better approximation for the NOE on H3, while methods **3** and **3'** (0.55 and 0.53, respectively) gave a better agreement for the NOE on H4.

Another datum to correlate with experimental values is the distance between the hydrogens involved in the glycosidic bond, *i.e.*, H1' and H3. Table 1 and Table 3 show this distance calculated by geometry considerations from angles ϕ and ψ (map averaging) or directly from PCModel (minima averaging) gives values of 2.25-2.27 Å by method **1**, 2.52-2.58 Å (method **2**, 2.65 Å (method **3**), 2.51 Å (method **2'**) and 2.62 Å (method **3'**). Though this distance has not been measured experimentally, it has been suggested[21] that it can be related with the chemical shift glycosylation displacement of the anomeric carbon (C1') in the ^{13}C NMR spectrum. For this disaccharide this displacement is +3.0 ppm,[22] leading Shashkov *et al.*[23] to infer that the global minimum for a disaccharide of this kind should be in the region that we call B, matching their energy plots.[23] The semiquantitative treatment of the relation of Bock *et al.*[24] points that 3.0 ppm correspond to a distance of *ca.* 2.7 Å,[24] close to the results obtained using methods **3** (2.65 Å) and **3'** (2.62 Å).

Either Λ, NOE or distance calculations actually imply many approximations, so it is difficult to conclude if electrostatic interactions should account in a lesser degree than shown in method **2**, or if they should be discarded at all (as in method **3**).

TABLE 3. Torsion angles (°), relative energies (kcal/ mol), populations (%), linkage rotations (Λ, in °), relative NOE values and H1'-H3 distances (Å) and for the optimal conformations obtained for disaccharide α-Gal-(1→3)-β-Gal using the MM2CARB force-field.

	Method 2'	Method 3'
Minimum A		
ϕ,ψ	-25, 57	-28, 58
E_{rel}	1.73	1.43
Population	4.6	8.6
Λ	-155	-150
NOE on H3[a]	0.52	0.53
NOE on H4[a]	-0.07	-0.07
$d_{H1'-H3}$	2.30	2.31
Minimum B		
ϕ,ψ	-41,-35	-46,-40
E_{rel}	0.00	0.00
Population	95.4	91.3
Λ	+43	+58
NOE on H3[a]	0.32	0.22
NOE on H4[a]	0.49	0.56
$d_{H1'-H3}$	2.52	2.65
Minimum C		
ϕ,ψ	-25,-174	-27,-175
E_{rel}	4.88	3.85
Population	0.02	0.1
Λ	-42	-40
NOE on H3[a]	0.00	0.01
NOE on H4[a]	0.04	0.03
$d_{H1'-H3}$	3.58	3.58
AVERAGE		
Λ	+ 34	+ 40
NOE on H3[a]	0.33	0.25
NOE on H4[a]	0.45	0.53
$d_{H1'-H3}$	2.51	2.62

a. By irradiating H1', relative to the NOE on H2'.

Actually, the possibility of considering electrostatic interactions in a lower degree may be made using a dielectric constant higher than the default (1.5) used by PCModel. Figure 3 shows that at a ε=10 (suggested for a water solution[5]) the effect is very similar to not considering electrostatic interactions at all. The analysis of the energy contributions which lead to this result are shown in Table 2, where it can be seen that minimum A is partly stabilized against minimum B when using a low ε, precisely by electrostatic forces, as the rest of contributions are almost constant with variations in ε. Table 2 also shows that using a distance-dependent ε (as used with AMBER[18]) produces only a small change in ΔE. On the other hand, considering hydrogen-bonding stabilizes sharply minimum A against B not only by these hydrogen bonds (included in the non-bonded interactions) but also by the electrostatic term (Table 2), either using dipole-dipole or charge-charge interactions.

Results obtained by HSEA calculations for this disaccharide[24] give a minimum-energy conformation at ϕ= -50°, ψ= -34° ($d_{H1'-H3}$= 2.65 Å), coincident with our region B.

MM2CARB gives similar results to those obtained with MM2. The values obtained by methods **2'** and **3'** as well as those obtained by methods **2** and **3** are close to the experimental values. It is noteworthy that even if *exo*-anomeric effect has not been explicitly accounted for in the force-field MM2, most of the allowed conformers (Fig.2a, b and c) either by MM2 or by MM2CARB are located in the ϕ g^- (-30 to -60°) band, which this stereo-electronic effect predicts.

EXPERIMENTAL

Calculations were made as described in the Procedure Section, on an 8086-based computer using PCModel 2.0 (Serena Software). The plots of the maps (Fig. 2) and the calculations of populations, average linkage rotation, atomic distance, *etc.* were carried out using the program DISMAP, written in Quick Basic 4.0 (Microsoft), under development in our lab, designed for that purpose. NOE theoretical calculations were performed as depicted by Imberty *et al.*[25]

ACKNOWLEDGEMENTS

Both authors are Research Members of the National Research Council of Argentina (CONICET). We are indebted to Drs. Oscar Varela and Gerardo Burton for helpful discussions, and to CONICET, TWAS and UNESCO for granting this work.

REFERENCES AND NOTES

1. A.D.French and J.W.Brady, *Computer Modeling of Carbohydrate Molecules*; A.D.French and J.W.Brady, Eds.; ACS Symposium Series; Washington DC, 1990, p 1.
2. R.U.Lemieux, K.Bock, T.J.Delbaere, S.Koto and V.S.Rao, *Can. J. Chem.*, **58**, 631 (1980).
3. H.Thøgersen, R.U.Lemieux, K.Bock and B.Meyer, *Can. J. Chem.*, **60**, 44 (1982). R.U.Lemieux and S.Koto, *Tetrahedron*, **30**, 1933 (1974). J.-P.Praly and R.U.Lemieux, *Can. J. Chem.*, **65**, 213 (1987).
4. G.A.Jeffrey, in *Computer Modeling of Carbohydrate Molecules* (see ref.1), p 20.
5. G.M.Lipkind, V.E.Verovsky and N.K.Kochetkov, *Carbohydr. Res.*, **133**, 1 (1984).
6. P.G.Goekjian, T-C.Wu and Y.Kishi, *J. Org. Chem.*, **56**, 6412 (1991).
7. A.D.French, R.S.Rowland and N.L.Allinger, in *Computer Modeling of Carbohydrate Molecules* (see ref.1), p 120. A.D.French, V.H.Tran and S.Pérez, *ibid.*, p 191.
8. I.Tvaroška and S.Pérez, *Carbohydr. Res.*, **149**, 389 (1986).
9. K.E.Gilbert and J.J.Gajewski, Indiana University, from N.L.Allinger, *J. Am. Chem. Soc.*, **99**, 8127 (1977); N.L.Allinger and Y.H.Yuh, *Quantum Chemistry Program Exchange* 395, Indiana University.
10. K.Morgan and A.N.O'Neill, *Can. J. Chem.*, **37**, 1201 (1959).
11. K.J.Lloyd, *Organic Chemistry. Series Two. Vol.7. Carbohydrates*; G.O.Aspinall Ed.; Butterworths Co.; 1976, p 252.
12. G.A.Jeffrey and R.Taylor, *J. Comput. Chem.* , **1**, 99 (1980).
13. IUPAC Tentative Rules for the Nomenclature of Organic Chemistry, *Eur. J. Biochem.*, **18**, 151 (1971).
14. V.H.Tran and J.W.Brady, *Biopolymers*, **29**, 961 (1990).
15. K.Rasmussen and J.Fabricius, *Computer Modeling of Carbohydrate Molecules* (see ref.1), p 177.
16. D.Lamba, A.L.Segre, S.Glover, W.Mackie, B.Sheldrick and S.Pérez, *Carbohydr. Res.*, **208**, 215 (1990).
17. K.Bock and R.U.Lemieux, *Carbohydr. Res.*, **100**, 63 (1982).
18. S.J.Weiner, P.A.Kollman, D.A.Case, U.C.Singh, C.Ghio, G.Alagona, S.Propheta, Jr. and P.Weiner, *J. Am. Chem. Soc.*, **106**, 765 (1984).
19. I.Tvaroška and T.Bleha, *Adv. Carbohydr. Chem. Biochem.*, **47**, 45 (1989).
20. D.A.Rees, *J. Chem. Soc. (B)*, 877 (1970); D.A.Rees and D.Thom, *J. Chem. Soc. Perkin Trans. II*, 191 (1977).

21. N.K.Kochetkov, O.S.Chizhov and A.S.Shashkov, *Carbohydr. Res.*, **133**, 173 (1984).

22. A.I.Usov, V.V.Barbakadze, S.V.Yarotskii and A.S. Shashkov, *Bioorg. Khim.*, **4**, 1507 (1978).

23. A.S.Shashkov, G.M.Lipkind, Yu.A.Knirel and N.K. Kochetkov, *Magn. Reson. Chem.*, **26**, 735 (1988).

24. K.Bock, A.Brignole and B.W.Sigurskjold, *J. Chem. Soc. Perkin Trans. II*, 1711 (1986).

25. A.Imberty, V.Tran and S.Pérez, *J. Comput. Chem.*, **11**, 205 (1989).