ELSEVIER

Carbohydrate Research 337 (2001) 2311–2323

CARBOHYDRATE RESEARCH

www.elsevier.com/locate/carres

Potential energy surfaces of carrageenan models: carrabiose, β-(1→4)-linked D-galactobiose, and their sulfated derivatives

Carlos A. Stortz*

Departamento de Química Orgánica-CIHIDECAR, Facultad de Ciencias Exactas y Naturales, UBA, Pab. 2 Ciudad Universitaria, 1428 Buenos Aires, Argentina

Received 22 March 2002; accepted 20 June 2002

Dedicated to Professor Derek Horton on the occasion of his 70th birthday

Abstract

The adiabatic conformational surfaces of several β-linked disaccharides, which correspond to the repeating structures of carrageenans, were calculated using the MM3 force-field. The studies were carried out on the disaccharide β-D-Gal*p*-(1→4)-α-D-Gal*p* and eight sulfated derivatives, as well as on carrabiose (β-D-Gal*p*-(1→4)-3,6-An-α-D-Gal*p*) and five sulfated derivatives. The presence of 3,6-anhydrogalactose does not change the main features of the maps, although it increases the flexibility of the glycosidic linkage. Sulfation neither produces a striking effect on the map shape, nor a shift on the global minimum, which always remains with ψ ($\theta_{C\text{-}1'\text{–}O\text{-}4\text{–}C\text{-}4\text{–}C\text{-}5}$) in trans orientation, and ϕ ($\theta_{O\text{-}5'\text{–}C\text{-}1'\text{–}O\text{-}4\text{–}C\text{-}4}$) with a value close to −80°. This effect differs from that occurring on the α linkage of equivalent disaccharides, for which the sulfation pattern on the β-galactose unit shifts the global minima to different positions. A reduction in the flexibility (originated in a deepening of the global minimum well) is observed by sulfation on position 2 of the β-D-galactose unit, and by sulfation of position 6 of the α-D-galactose unit (when the β-D-galactose unit is 4-sulfated). Within the compounds containing 3,6-anhydrogalactose, the effect of sulfation is even less noticeable. The calculated low-energy regions on carrabiose derivatives agree with X-ray diffraction data on carrageenan fibers and on peracetylated carrabiose dimethyl acetal, and with NOE calculations carried out on κ-carrabiose.

Keywords: Conformational analysis; Disaccharide maps; Carrabiose; MM3; Carrageenans

1. Introduction

Carrageenans are sulfated galactans that can be extracted from red seaweeds for use as thickeners and gelling agents. Those galactans have structures based on linear chains of alternating 4-linked α-D-galactopyranosyl (A) residues and 3-linked β-D-galactopyranosyl (B) residues, usually sulfated in different positions, and having often the α-D-galactose unit replaced by 3,6-anhydrogalactose.[1] The useful physical properties of these polysaccharides depend on their conformations and molecular flexibility, which is concentrated mostly on the glycosidic linkages. Thus, conformational analysis of the various disaccharides that act as repeating units should be useful in improving the understanding of the physical and biological properties of those macromolecules.

Conformational studies of sulfated sugars were formerly hindered because the compounds were difficult to crystallize for diffraction crystallography and the necessary parameters for molecular mechanics modeling calculations were lacking. In the past few years, however, parameters for sulfated carbohydrates using different modeling force-fields[2–8] have been developed based on ab initio calculations and on X-ray studies. Fiber diffraction studies of several oriented carrageenan fibers have been carried out,[9–14] as well as single-crystal X-ray diffraction studies of neocarrabiose[15] (3,6-An-α-D-Gal*p*-(1→3)-β-D-Gal*p*) and the peracetylated dimethyl acetal of carrabiose[16] (β-D-Gal*p*-(1→4)-3,6-An-α-D-Gal). Modeling studies of carrabiose and some of its

* Tel./fax: +54-11-4576-3346
E-mail address: stortz@qo.fcen.uba.ar (C.A. Stortz).

0008-6215/02/$ - see front matter
PII: S0008-6215(02)00173-8

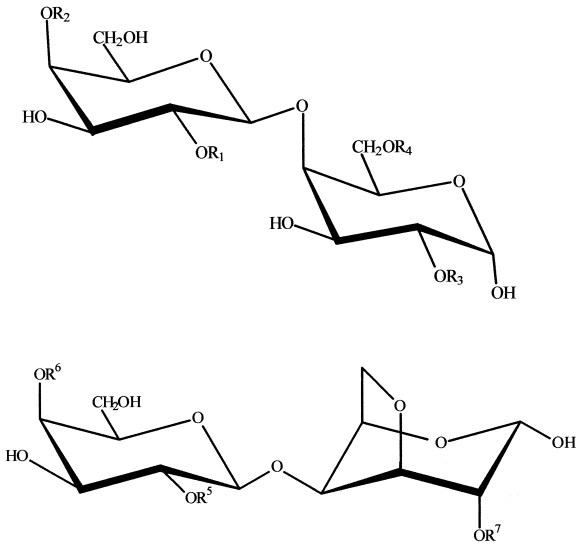
sulfated derivatives, which are the repeating units of β-, κ- and ι-carrageenan, have been carried out with several methods that used either rigid or flexible monomeric residues,[8,17–19] Other disaccharides found in carrageenans, such as neocarrabiose,[19–21] α-D-Gal*p*-(1→3)-β-D-Gal*p*,[22–25] and their sulfated derivatives,[8,18,21,23,24] have been modeled by molecular mechanics and also by molecular dynamics.[19,20]

MM3[26,27] is considered a useful force-field for carbohydrates, given its considerations to hydrogen-bond forces and anomeric and exo-anomeric effects.[28] Among others, Dowd et al. applied this force-field to evaluate the potential energy surfaces of many disaccharides.[29–32] In previous papers, we have presented the conformational features of the AB repeating units of carrageenans α-D-Gal*p*-(1→3)-β-D-Gal*p*,[22,24,25] neocarrabiose,[21] and 17 of their sulfated derivatives,[21,24] based on studies with the MM3 force-field parameterized for sulfate as described by Lamba et al.[7] Herein, we show the conformational energy surfaces of the BA units: nine derivatives of β-D-Gal*p*-(1→4)-α-D-Gal*p* and six derivatives of carrabiose (Fig. 1), calculated with the same force-field. By comparing these new maps with the previous ones, the influence of the 3,6-anhydro ring and the sulfate groups on the molecular conformation of both disaccharidic repeating units of carrageenans was determined.

2. Methods

The molecular mechanics program MM3 (92) (QCPE, Indiana University, USA), developed by Allinger and co-workers was used.[26,27] However, the MM3 routines were modified as suggested[33] by changing the maximum atomic movement from 0.25 to 0.10 Å. The dihedral angles ϕ and ψ are defined by atoms O-5′–C-1′–O-4–C-4 and C-5–C-4–O-4–C-1′, respectively, with the usual sign conventions.[21] Other papers dealing with carrabiose conformations[10,16,18,19] have used other definitions for these angles. On the other hand, ϕ_H and ψ_H are defined by atoms H-1′–C-1′–O-4–C-4 and H-4–C-4–O-4–C-1′, respectively. In our previous work, we have used exclusively those ϕ_H,ψ_H hydrogen-related angles.[21–25] However, it has been suggested[34] that it is better to drive torsion angles in terms of non-hydrogen atoms, given the different motions of the three atoms during driven rotation and the inaccuracy of hydrogen atom positions in diffraction studies. The orientation of the hydroxyl hydrogens is indicated by χ_n, defined by the atoms H-*n*–C-*n*–O-*n*–H(O)-*n*, while χ_6 is defined by the atoms C-5–C-6–O-6–H(O)-6, and ω by the atoms O-5–C-5–C-6–O-6. Their values are described by one of these eight one-letter codes:[35] **S** for angles between −30 and +30°, **g** for 30–80°, **p** for 80–100°, **e** for 100–150°, **T** for 150–210°, **E** for 210–260°, **P** for 260–280°, and **G** for 280–330°. When a sulfate group replaced a hydroxyl hydrogen, the same definition was used.

MM3 parameters for the sulfate group were taken from Lamba et al.,[7] and a dielectric constant of 3.0 was used. In that model, the charge on the sulfate groups is emulated by S–O bond dipoles. No cations were added. To generate each map, for each compound, ca. 25–30 conformers in different regions of the ϕ,ψ space with varied exocyclic groups orientation were chosen as starting points. Those conformers were found using an iterative method.[21,22,25] Minimization was carried out



	R^1	R^2	R^3	R^4	Repeating unit of carrageenan
1	H	H	H	H	
2	SO_3^-	H	H	H	
3	H	SO_3^-	H	H	
4	SO_3^-	H	SO_3^-	H	ξ–
5	H	SO_3^-	SO_3^-	H	
6	SO_3^-	H	H	SO_3^-	
7	H	SO_3^-	H	SO_3^-	μ–
8	SO_3^-	H	SO_3^-	SO_3^-	λ–
9	H	SO_3^-	SO_3^-	SO_3^-	ν–

	R^5	R^6	R^7	
10	H	H	H	β-
11	SO_3^-	H	H	
12	H	SO_3^-	H	κ-
13	H	H	SO_3^-	α-
14	SO_3^-	H	SO_3^-	Alkali treated λ-
15	H	SO_3^-	SO_3^-	ι-

Fig. 1. The disaccharides used in this work.

by the block diagonal Newton–Raphson procedure for grid points and using the full-matrix procedure for minima. Using both the dihedral drivers 2 and 4, ϕ and ψ were fully varied using a 20° grid. At each point, energies were calculated after minimization with restraints for these two angles, but allowing the other variables to relax. The optimization was terminated when the decrease in energy converged to a value lower than 2 cal/mol. The energy for each point was the lowest of any of the 25–30 different minima obtained previously. In this way, only the conformation of minimal energy for each ϕ,ψ combination was recorded. The conformational adiabatic maps, or energy surfaces as function of ϕ and ψ angles were produced. Free energies were calculated from the vibrational analysis of the minima, with no special treatment for the low-frequency vibrations:[36] i.e., the effect of frequencies equal or lower than 20 cm^{-1} was added to the MM3 output values of vibrational enthalpies and entropies.

The absolute flexibility was calculated as described by Koča and co-workers[28,37] First, the energies and geometries of the transition states between minimum energy regions were calculated: they were first estimated from the walk within the adiabatic maps, and then determined by a full-matrix analysis, confirming that only one negative eigenvalue appeared. When more than one transition state was present in the same region, that with lower energy was considered. Then, the absolute flexibility[28,37] Φ was calculated as:

$$\Phi = \sum_{i=1}^{n}\left(\frac{e^{-E_i/RT}}{\sum_{k=1}^{n} e^{-E_k/RT}}\right) \times \left[\sum_{j=1}^{m}(e^{-(E_j-E_{gm})/RT}) \times \left(\frac{|\phi_i-\phi_j|+|\psi_i-\psi_j|}{720}\right)\right]$$

where E_{gm} is the energy of the global minimum, n is the number of minima (indexes i and k), m the number of transition states (index j) surrounding minimum i, the ϕ,ψ angles are given in degrees, R is the universal gas constant and T is the temperature (set to 25 °C = 298.16 K).

The partition function was calculated as:[21]

$$q = \Delta\phi \times \Delta\psi \times \sum_{i=1}^{324} e^{-(E_i-E_{gm})/RT}$$

where $\Delta\phi$ and $\Delta\psi$ are the grid spacings (20° each in this case) and the summation is carried out over the entire ϕ,ψ surface (324 points). French and co-workers[38] used the name "probability volume" for this function.

3. Results

The conformational map of the disaccharide β-D-Gal*p*-(1→4)-α-D-Gal*p* (**1**) calculated using MM3 was obtained. This force-field, specially parameterized for sulfated carbohydrates, was also used for the study of some sulfated derivatives: in compound **2** the hydroxyl group at C-2 of the β-galactose unit was sulfated, and in compound **3**, the hydroxyl group at C-4 of the same unit was sulfated. Calculations were also carried out with disaccharide **4** (sulfated on both positions 2), and on compound **5**, sulfated on O-2 of α-galactose and on O-4 of β-galactose, and also on disaccharides **6** (sulfated on O-6 of α-galactose and on O-2 of β-galactose) and **7** (sulfated on O-6 of α-galactose and on O-4 of β-galactose, repeating unit of μ-carrageenan). Finally, the maps of compounds **8** and **9**, which carry an additional sulfate on O-6 of the α-galactose unit with respect to **4** and **5**, were also calculated. Structures **8** and **9** are the repeating units of λ- and ν-carrageenans, respectively. The resulting maps are shown in Figs. 2 (**1**), 3 (**2**, **4**, **6** and **8**) and 4 (**3**, **5**, **7** and **9**), while the geometric and energy data (steric and free energy) on the minima are shown in Table 1.

All the maps have very similar shapes (Figs. 2–4), with the global minimum in the **A** region (around $\phi,\psi = -80°,140°$), and other three minima. The **B** minimum is very close in energy and geometry to the global one, appearing around $\phi,\psi = -130°,110°$, but shifting to $\phi,\psi = -120°,80°$ for the compounds sulfated on position 2 of the β-galactose unit (Table 1). The minimum **C** is located on the same ϕ trough, with a ψ angle close to $-70°$. Finally, a fourth minimum in the **D** region, with a ϕ value shifted in 180° from those of the **A**–**B**–**C** minima appears with energies 2.5–6 kcal/mol above the global minimum. This region was

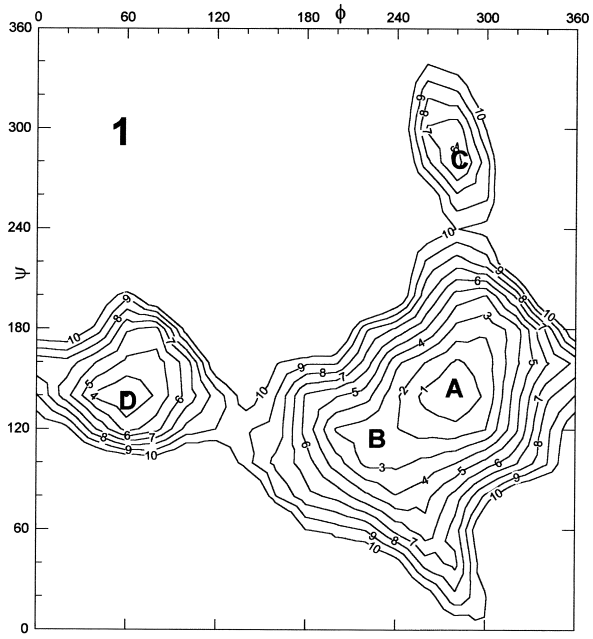


Fig. 2. Conformational maps of compound **1**, generated using MM3. Iso-energy contour lines are graduated in 1 kcal/mol increments above the global minimum.

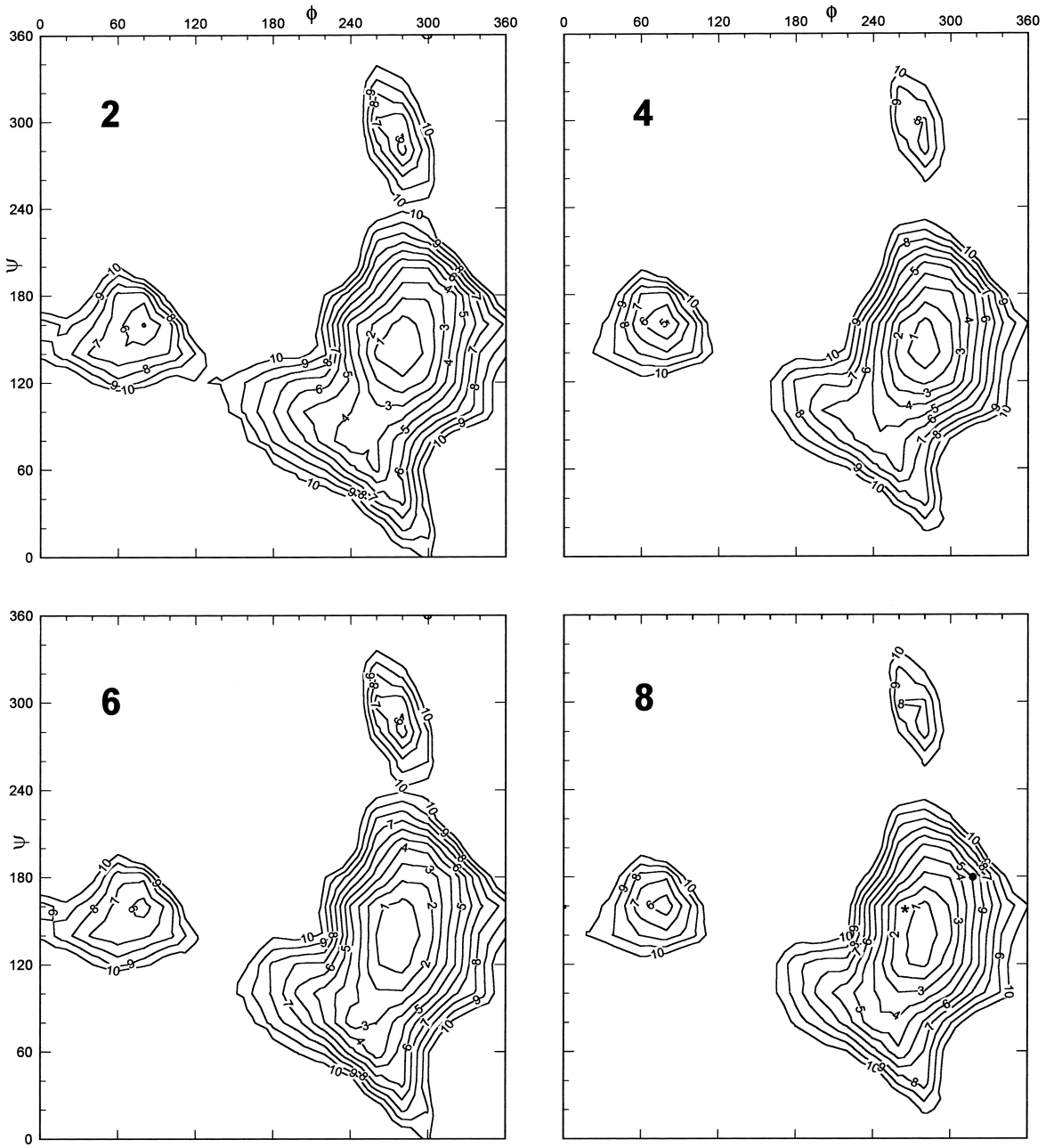


Fig. 3. Conformational maps of compounds **2**, **4**, **6** and **8**, generated using MM3. Iso-energy contour lines are graduated in 1 kcal/mol increments above the global minimum. On compound **8** the star and the black dot represent the geometries of the global minimum (Tripos force-field) and the expected helical conformation calculated by Le Questel et al.,[8] respectively.

also observed with the corresponding α-linked disaccharides, but its energy was very high.[22] Most of the lowest energy structures in each region had their hydroxymethyl groups in a GT ($\omega \approx 60-70°$) orientation, although, when a sulfate group on O-6 was present, the exocyclic group was sometimes forced to a GG ($\omega \approx -60°$) orientation.

The conformational map of carrabiose (β-D-Gal*p*-(1→4)-3,6-An-α-D-Gal*p*, **10**) was also calculated with the same procedure, as well as those for some sulfated derivatives: in compound **11** the hydroxyl group at C-2 of the β-D-galactose unit was sulfated, and in compound **12**, the hydroxyl group at C-4 of the same unit was sulfated (repeating unit of κ-carrageenan). Calculations were also carried out with the same compounds, sulfated on position 2 of the 3,6-anhydrogalactose unit (disaccharides **13**, **14** and **15**, repeating units of α-, alkali-treated λ-, and ι-carrageenans, respectively). The resulting maps are shown in Fig. 5, while the geometric and energy data on the minima are shown in Table 2.

All the maps (Fig. 5) have shapes similar as those of

the non-3,6-anhydro counterparts **1**–**9** (Figs. 2–4). Again, the global minimum was in the **A** region (around $\phi,\psi = -80°,170°$), and the maps exhibit the other three minima before mentioned. However, the **B** minimum appears better defined, closer in energy, but farther in geometry to the global one. The allowed surface (to 10 kcal/mol) appears larger (cf. Figs. 2–4 with Fig. 5). Besides, in the **D** region several minima (with $\phi,\psi \approx 50°,150°$, $\phi,\psi \approx 80°,180°$, and $\phi,\psi \approx 60,120°$) appear in all compounds but **11**. In Table 2 only those with the lowest strain energy are shown. All of the low-energy structures in each region had their hydroxymethyl group in a GT ($\omega \approx 60–70°$) orientation.

The conformational partition functions (probability volumes),[38] absolute flexibilities and potential barriers between minima **A** and **B** are shown on Table 3 for disaccharides **1**–**15**. Besides, the same functions were calculated for their equivalent disaccharides with α-(1→3)-linkage (AB disaccharides), for which maps were previously determined.[21,24] Table 4 shows the main hydrogen bond arrangements determined for each of the four minima in compounds **1**–**15**.

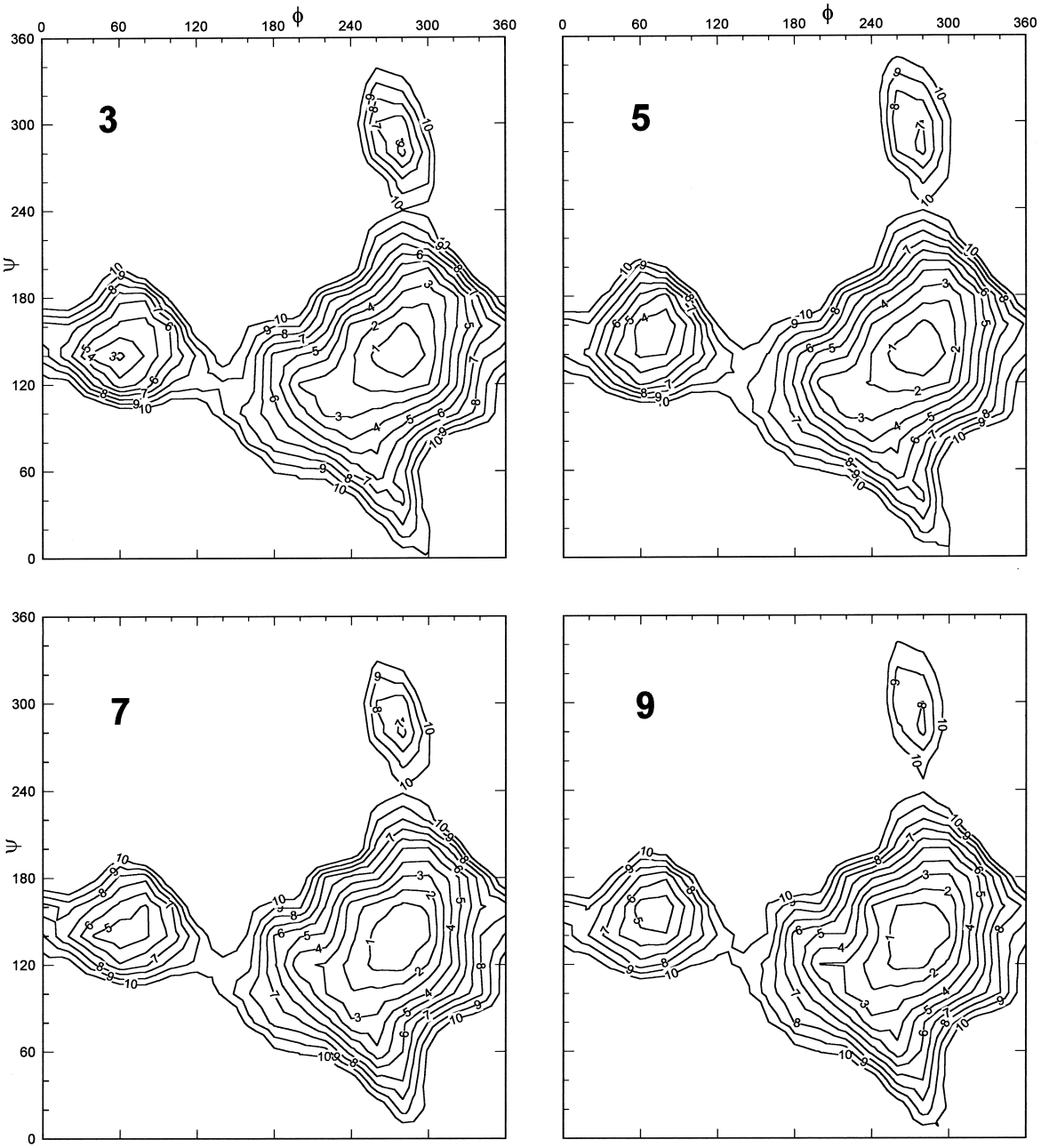


Fig. 4. Conformational maps of compounds **3**, **5**, **7** and **9**, generated using MM3. Iso-energy contour lines are graduated in 1 kcal/mol increments above the global minimum.

Table 1
Torsion angles (°), relative strain energies and free energies (kcal/mol) and exocyclic angles for the minimum-energy conformations [a] obtained for sulfated derivatives of the disaccharide β-D-Gal*p*-(1→4)-α-D-Gal*p*, using the MM3 force-field

		ϕ,ψ	ϕ_H,ψ_H	E_{rel} (G_{rel})	Exocyclic torsion angles [b]			
					$\chi_{2'}\chi_{3'}\chi_{4'}$	$\omega\chi_{6'}$	$\chi_1\chi_2\chi_3$	$\omega\chi_6$
1	Region A	−80,142	42,23	0.00 (0.00)	Ggg	gG	GTg	gG
	Region B	−134,112	−15,−10	2.13 (2.44)	Ggg	gG	GTg	gG
	Region C	−84,−71	39,173	5.55 (6.87)	Ggg	gT	ggT	gG
	Region D	57,136	174,15	2.89 (4.93)	Ggg	gT	ggT	Gg
2	Region A	−80,144	41,25	0.00 (0.00)	Sgg	gG	GTg	gG
	Region B	−117,80	4,−44	3.11 (4.05)	Sgg	gG	ggT	gG
	Region C	−84,−71	38,173	5.25 (6.87)	Sgg	gT	ggT	gG
	Region D	79,163	−165,45	4.87 (6.40)	Sgg	gT	ggT	gG
3	Region A	−80,142	42,22	0.00 (0.00)	GgS	gG	GTg	gG
	Region B	−133,112	−15,−9	2.16 (2.35)	GgS	gG	GTg	gG
	Region C	−84,−72	38,173	5.38 (7.00)	GgS	gT	ggT	gG
	Region D	57,136	174,15	2.62 (4.71)	GgS	gT	ggT	Gg
4	Region A	−81,144	40,25	0.00 (0.00)	Sgg	gG	gSg	gG
	Region B	−123,92	−3,−32	4.94 (4.36)	Sgg	gG	gSg	gG
	Region C	−87,−69	35,175	7.14 (8.04)	Sgg	gP	gST	gG
	Region D	77,162	−167,44	4.75 (5.98)	Sgg	gP	gST	gG
5	Region A	−80,141	41,22	0.00 (0.00)	GgS	gG	gSg	gG
	Region B	−135,112	−17,−10	1.96 (2.45)	GgS	gG	gSg	gG
	Region C	−87,−70	35,174	6.30 (7.78)	GgS	gG	gST	gG
	Region D	79,160	−164,42	3.21 (5.52)	gGS	gP	gST	gG
6	Region A	−85,148	36,28	0.00 (0.00)	Sgg	gG	GTg	GT
	Region B	−114,79	7,−45	2.46 (4.46)	Sgg	gG	ggT	gE
	Region C	−85,−71	37,173	5.11 (7.05)	Sgg	gT	ggT	gT
	Region D	78,164	−165,47	6.03 (8.04)	Sgg	GG	ggT	gP
7	Region A	−85,145	36,25	0.00 (0.00)	GgS	gG	GTg	GT
	Region B	−152,117	−34,−4	2.95 (4.11)	GgS	gG	GTg	Ge
	Region C	−84,−71	38,173	6.35 (6.43)	GgS	gT	ggT	gE
	Region D	56,137	173,16	4.12 (5.23)	GgS	gT	ggT	Ge
8	Region A	−85,147	36,27	0.00 (0.00)	Sgg	gG	gSg	GT
	Region B	−112,85	9,−39	3.77 (4.92)	Sgg	gG	GSg	gP
	Region C	−87,−69	35,175	7.03 (8.62)	Sgg	gP	gST	gT
	Region D	77,162	−167,44	5.87 (7.14)	Sgg	gP	gST	gP
9	Region A	−88,144	33,24	0.00 (0.00)	GgS	gG	GSg	GT
	Region B	−152,117	−35,−4	2.82 (4.89)	GgS	gG	gSg	Ge
	Region C	−88,−69	34,175	7.32 (8.31)	GgS	gP	gST	gE
	Region D	79,160	−164,43	4.36 (6.92)	gGS	gP	GST	gG

[a] When the strain energy minimum and the free energy minimum structures are not the same, data shown corresponds to the strain energy minimum.
[b] For nomenclature, see Methods.

4. Discussion

Previous papers[22–24] showed that the conformational maps around the glycosidic bonds of α-D-galactopyranosyl-(1→3)-β-D-galactopyranose and its sulfated derivatives have three main energy minima, suggesting substantial conformational flexibility for the glycosidic linkage. The three minima occur in a trough with an almost constant ϕ_H in near the g^- conformation as an expected expression of the exo-anomeric effect. Usually the minimum called **B** (with ψ_H in near g^- conformation) was the global minimum, with small energy differences with the so-called minimum **A** (in which ψ_H has positive values). Sulfation on the β-D-galactose unit led to a deepening of the well at the **B** region when occurs on position 2, whereas it shifts the global minimum to the **A** region when occurs on position 4. This effect agrees with the expectations from the ^{13}C NMR chemical shifts of the polysaccharides containing those repeating units.[24] Further work, in which the

α-D-galactose unit has been replaced by its 3,6-anhydro derivative, showed similar maps and sulfation effects, although a greater flexibility of the glycosidic linkage was also observed.[21]

The potential energy surface of β-D-galactopyranosyl-(1→4)-α-D-galactopyranose (Fig. 2) shows two perpendicularly intersecting low-energy troughs, as occurs with other β-linked disaccharides studied with the same force-field[29] and also with the corresponding C-disaccharides.[39] The trough aligned with the ψ-axis shows ϕ_H in a near-g^+ conformation, the expected expression of the exo-anomeric effect, while the perpendicular trough is centered on an eclipsed ψ_H angle. Minimum **A**, which is close to the crossing of both troughs ($\phi,\psi = -80°,142°$; $\phi_H,\psi_H = 42°,23°$), is the global one. Very close in geometry, minimum **B** is 2 kcal/mol less stable (Table 1). Minimum **C**, within the same vertical trough is much less stable, even less than for the α-linked compounds,[24] while a ''horizontal'' walk gives rise to a low-energy minimum **D**, about 3 kcal/mol above the global one.

The flexibility of the glycosidic linkage has been measured in terms of a partition function[21] (probability volume[38]), which is highly dependant on the energy differences between the minima **A** and **B**, as its magnitude is very sensitive to the size of the regions of the map with very low-energy.[40] Thus it is highly influenced by the entropy of the global minimum. Another parameter, which has been applied to carbohydrates,[28,37,40] is the ''absolute flexibility'', which gives an indication of the conformational interconversions of the lower energy minima, and is very sensitive to the height of their lower potential barriers. A marked increase in flexibility (by either parameter) is observed when passing from the disaccharides containing α-galactose to those containing 3,6-anhydrogalactose (Table 3). It was previously predicted that equatorially linked disaccharides are more flexible than those carrying at least one axial bond.[41] Such a trend was shown to hold in many cases,[38,40] and can explain the enhanced flexibility: for the non-3,6-anhydro disaccharide (α-D-galactose with

Table 2
Torsion angles (°), relative strain energies and free energies (kcal/mol) and exocyclic angles for the minimum-energy conformations [a] obtained for sulfated derivatives of the disaccharide β-D-Gal*p*-(1→4)-3,6-An-α-D-Gal*p*, using the MM3 force-field

		ϕ,ψ	ϕ_H,ψ_H	E_{rel} (G_{rel})	Exocyclic torsion angles [b]		
					$\chi_{2'}\chi_{3'}\chi_{4'}$	$\omega\chi_{6'}$	$\chi_1\chi_2$
10	Region A	−81,168	40,44	0.00 (0.00)	Ggg	gG	GG
	Region B	−91,77	31,−51	0.84 (0.59)	Ggg	gG	GG
	Region C	−72,−46	52,−169	6.77 (8.14)	Ggg	gG	GG
	Region D	77,−176	−166,62	4.50 (5.06)	Ggg	gG	GG
11	Region A	−84,168	37,44	0.00 (0.00)	Sgg	gG	GG
	Region B	−92,77	29,−51	1.24 (1.00)	Sgg	gG	GG
	Region C	−72,−46	51,−169	7.25 (8.57)	Sgg	gG	GG
	Region D	55,157	173,33	4.81 (5.56)	Sgg	gG	GG
12	Region A	−81,168	40,44	0.00 (0.00)	GgS	gG	GG
	Region B	−90,78	32,−50	0.89 (0.53)	GgS	gG	GG
	Region C	−72,−47	52,−170	6.71 (8.22)	GgS	gG	GG
	Region D	81,−176	−162,62	3.65 (4.39)	gTS	gG	GG
13	Region A	−79,169	43,45	0.00 (0.00)	Ggg	gG	TS
	Region B	−90,78	32,−51	1.12 (0.95)	Ggg	gG	TS
	Region C	−71,−47	52,−170	6.85 (8.40)	Ggg	gG	TS
	Region D	46,146	165,20	3.05 (4.98)	Ggg	gP	TS
14	Region A	−83,169	38,45	0.00 (0.00)	Sgg	gG	TS
	Region B	−94,77	27,−52	1.23 (1.00)	Sgg	gG	TS
	Region C	−72,−47	51,−170	7.21 (8.69)	Sgg	gG	TS
	Region D	46,151	164,25	3.21 (5.82)	Sgg	gP	TS
15	Region A	−78,170	43,45	0.00 (0.00)	GgS	gG	TS
	Region B	−93,76	28,−52	1.15 (2.26)	gTS	gG	TS
	Region C	−71,−47	52,−170	6.71 (8.37)	GgS	gG	TS
	Region D	84,−177	−159,61	2.15 (4.83)	gTS	gG	TS

[a] When the strain energy minimum and the free energy minimum structures are not the same, data shown corresponds to the strain energy minimum.

[b] For nomenclature, see Methods.

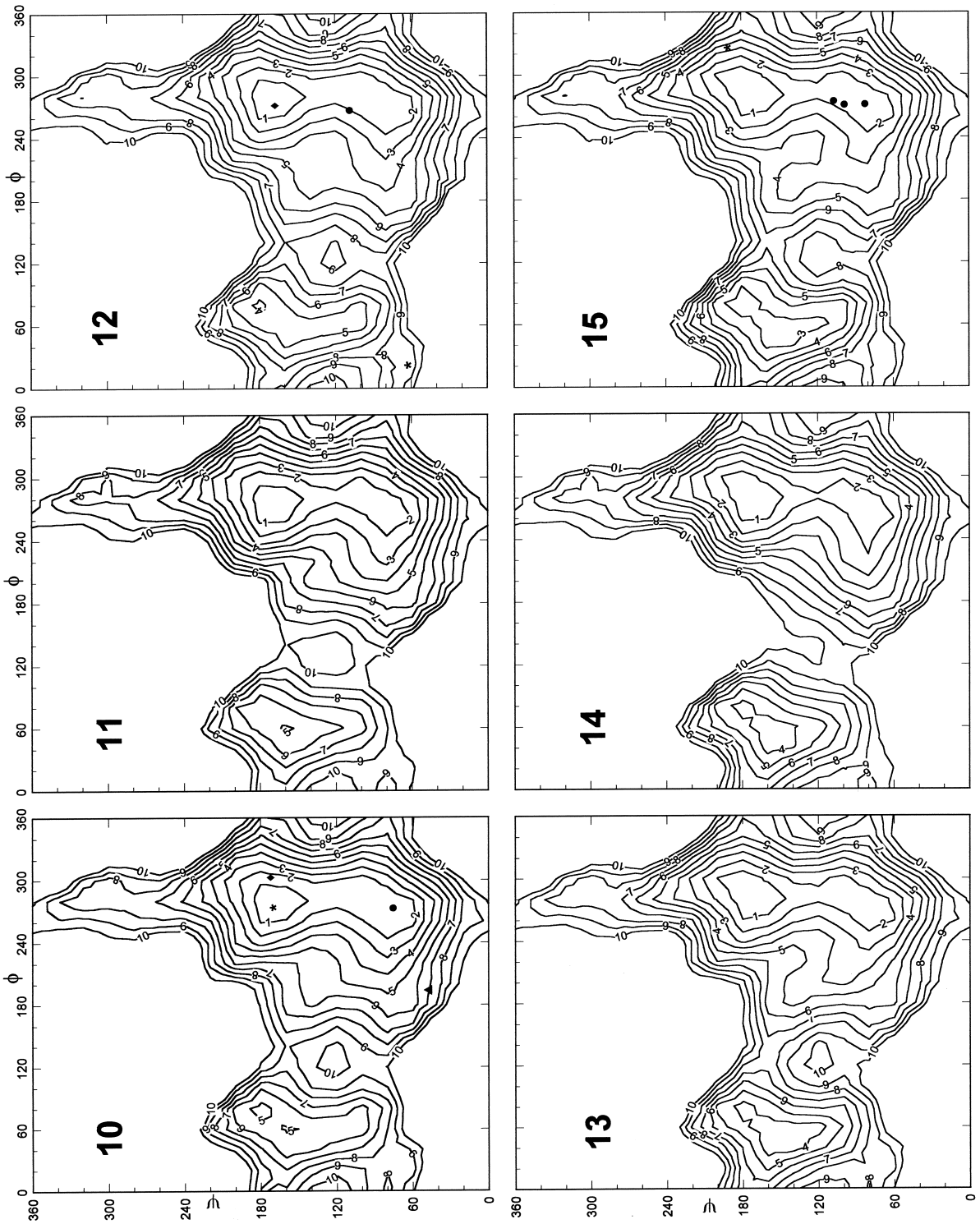


Fig. 5. Conformational maps of compound **10**–**15,** generated using MM3. Iso-energy contour lines are graduated in 1 kcal/mol increments above the global minimum. On compound **10** the symbols indicate the published crystal[16] structure (●); geometries calculated by Parra et al.[17](*) and by Ueda et al. [19] by molecular mechanics (◆) and molecular dynamics (▲). On compound **12** the symbols indicate the geometry of the published fiber[10–12] structure (●), and those calculated by Urbani et al.[18] (◆) and by Le Questel et al.[8] (*), while on compound **15** the symbols represent the geometries of different fiber[10,13,14] structures of *ι*-carrageenan (●), and calculated by Le Questal et al.[8] (*).

Table 3
Conformational partition functions q (deg^2), absolute flexibilities Φ ($\times 10^4$) and lowest potential barriers E_{barr} ($E_{TS}-E_{gm}$) between minima (kcal/mol) for **1**–**15**, using the MM3 forcefield. For comparison purposes, the same data for the equivalent (1→3)-linked AB units is given [a]

Disaccharide	BA			AB		
	q	Φ	E_{barr}	q	Φ	E_{barr}
1	880	27	2.2	1510	23	2.0
2	860	2.0	3.7	870	1.8	3.7
3	890	25	2.2	570	8.1	2.2
4	610	0.3	4.9	700	1.1	4.0
5	980	32	2.0	730	11	2.1
6	920	8.1	2.9	870	0.6	4.4
7	1140	1.1	3.9	530	2.5	3.5
8	660	1.6	3.9	850	0.4	4.6
9	1170	3.1	3.3	590	5.0	3.2
10	1210	68	1.4	2140	258	0.5
11	1050	43	1.7	1040	6.8	3.0
12	1200	65	1.4	1320	143	0.9
13	1330	44	1.7	2100	226	0.4
14	1290	33	1.9	1090	8.7	2.7
15	1430	42	1.7	1780	175	0.7

[a] The data for the AB units was recalculated from the maps given at Refs. 21 and 24, corrected when necessary.

$^4C_1$ conformation), the glycosidic oxygen is connected by an equatorial (C-1′) and an axial (C-4) bond, while in carrabiose (the 3,6-anhydrogalactose takes the $^1C_4$ conformation) both bonds become equatorial. The same effect occurs with the α-linkage[21] and also explains its enhanced flexibility (Table 3). A concomitant factor leading to an increased flexibility is the change of the C-3 substituent from the equatorial to the axial position. Bulky equatorial substituents vicinal to the glycosidic bond are also known to decrease the flexibility.[21,40,41]

Sulfation does not change the gross features of the map. Table 5 shows the (grossly additive) effects of sulfation on the energy differences between minima. It is shown that sulfation on position 4 of the β-D-galactose unit has almost no effect on relative energies and flexibilities (Tables 1 and 3, cf. **1** and **3**), as expected from its remoteness from the linkage. In those 4′-sulfated disaccharides, sulfation on position 2 of the α-D-galactose unit showed neither effect (Tables 1 and 3, cf. **3** with **5**, and **7** with **9**), but sulfation on position 6 of this unit lowers the stability of the **B** and **D** conformers. Therefore, the simultaneous presence of sulfate groups on O-4′ and O-6 produce a decrease in flexibility as computed by Φ (given its higher energy barrier), as well as an increase for the partition function, due to the decreased free energy of minimum **A** (Table 1). The potential energy surfaces of the 4′-sulfated compounds (Fig. 4) are very similar to those of the non-sulfated disaccharide (Fig. 2). The presence of a sulfate group on position 2 of the β-D-galactose unit also lowers the stability of the **B** and **D** conformers. This can be rationalized on the basis of the loss of the hydrogen bonding interactions of H(O)-2′ with O-6 (in minimum **B**) and with O-3 (in minimum **D**, see Table 4). The flexibility indicated by the Φ parameter is also decreased (Table 3), as expected for a bulky equatorial substituent vicinal to the glycosidic bond.[21,40,41] This effect is enhanced by a sulfate on O-2, and decreased by a sulfate on O-6 (Table 3), as expected from their effect on the relative energies of minima **A** and **B** (Table 5). The decrease in stability of the **D** minima is clearly observed in the maps (Fig. 3), as the **D** region appears as an ''island'' in the 10 kcal contour. A further decrease of the **D** stability is produced by sulfation on O-6 (Table 5, Fig. 3). On the other hand, sulfation on O-2 produces a clear destabilization of minima **B** and **C**, which cannot be explained in terms of hydrogen bonding (Table 4). As explained earlier, sulfation on positions 2 and 4 of the β-D-galactose unit had counteracting effects on the relative stabilities of the minima **A** and **B** when considering the α-linkage.[24] These effects are also shown on the last column of Table 5 and are easily explained on the basis of the proximity of those groups to that glycosidic linkage. As expected, the present work shows that the effects of sulfation on the relative energies of **A** and **B** for the β linkage are smaller and mostly due to sulfation on the closer hydroxyl groups on C-2′ and C-6. Sulfation on C-2 has also an effect on this difference only when C-2′ is also sulfated (Tables 1 and 5).

Replacement of the α-galactose unit by a 3,6-anhydro ring (compound **10**) does not change either the overall shape of the map. However, a quick observation indicates that the allowed surface below 10 kcal is greatly increased (Fig. 5). On the other hand, the minimum **A** appears now clearly differentiated from the **B** one (with a 100° difference in ψ), but with an energy difference lower than 1 kcal/mol (Table 2) and an energy barrier between them of only 1.4 kcal/mol (Table 3). As explained earlier (see Results section), in the **D** region three different minima were found. The influence of sulfation on the β-unit on the relative energies is negligible (Table 5, Fig. 5, cf. **10**, **11** and **12**). Previous work[21] has shown a marked influence of sulfation on position 2, and a minor one (of inverse sign) on position 4 on the relative stabilities of the two main minima when considering the α linkage (AB maps, Table 5), which has also reflected in the flexibilities (Table 3). Moreover, sulfation on position 2 of the 3,6-anhydrogalactose unit has a negligible effect on the relative stabilities of minima **A**, **B** and **C**, but has a stabilizing effect on minima **D** (Table 2 and 5), which can be rationalized on the basis of the stabilizing effect of the

hydrogen bond between H(O)-6′ and O(S)-2 (Table 4). On the α linkage, sulfation on position 2 of the 3,6-anhydrogalactose unit had only a significant effect on the relative energies of the main minima when the β-D-galactose unit is 4-sulfated.[21] The derivatives of carrabiose **10**–**15** showed almost constant values of flexibility (Table 3). A small increase in the partition function values are produced by C-2 sulfation, and a small decrease by C-2′ sulfation. Both C-2 and C-2′ sulfation produce small decreases in the absolute flexibilities, given their higher (+0.3 kcal/mol) potential barriers. Their α-linked counterparts, neocarrabiose and derivatives, are more flexible (Table 3) only when the β-D-galactose unit is not sulfated or sulfated on position 4.

The arrangements of hydrogen bonds (Table 4) may help to explain the relative stabilities of some minima (see above), but many cannot be explained by this factor. The need of calculating free energies was discussed in previous papers.[21,42] MM3 (94) free energy calculations for carbohydrates were validated by comparison with ab initio molecular orbital calculations.[43] The effect of calculating free energies has not changed the overall conclusions made previously, but has allowed us to determine some trends: (a) for compounds carrying 3,6-anhydrogalactose (but for **15**) minimum **A** is still the global minimum, but the relative free energy of **B** is lower (Table 2), while for the non-3,6-anhydro compounds **1**–**9**, minimum **A** appears even more stabilized than **B** (Table 1); and (b) minima **C** and **D** are less favored when free energies are calculated in all the instances, as occurred with the equivalent α-linked disaccharides.[21]

The quality of the fiber diffraction patterns for λ-carrageenan[44] is too poor for structure determination.[8,12] The geometric features of the helices calculated for this polysaccharide[8] yield ϕ,ψ values of −50°,180°, i.e., in the **A** region. The rigid-residue analysis of Le Questel et al.,[8] also gave the global minimum in the **A** region (Fig. 3), although their maps showed reduced flexibility and a different shape of the minimum energy region. For compounds containing 3,6-anhydrogalactose, more studies have been carried out: the X-ray crystal structure for peracetylated carrabiose dimethyl acetal[16] shows ϕ,ψ angles of −92°,72°, i.e., very close to our minimum **B** (ϕ,ψ = −91°,77°), for which the

Table 4
Hydrogen bond arrangements with energies higher than 0.6 kcal/mol, established in each minimum energy region for the fifteen compounds under study

	All minima	Min. A	Min. B	Min. C	Min. D
1			H(O)-2′–O-3	H(O)-3–O-5′	H(O)-3–O-5′ and H(O)-2′–O-6
2				H(O)-3–O-5′ and H(O)-3′–O(S)-2′	H(O)-3–O-5′ and H(O)-3′–O(S)-2′
4	H(O)-3′–O(S)-2′	H(O)-3–O(S)-2	H(O)3–O(S)2	H(O)-3–O-5′ and H(O)-6′–O(S)-2	H(O)-3–O-5′ and H(O)-6′–O(S)-2
6	H(O)-3′–O(S)-2′	H(O)-6′–O(S)-6	H(O)6′–O(S)6 and H(O)3–O(S)2′	H(O)-3–O-5′	H(O)-3–O-5′ and H(O)-6′–O-4′
8	H(O)-3′–O(S)-2′	H(O)-3–O(S)-2	H(O)3–O(S)2 and H(O)6′–O(S)6	H(O)-3–O-5′ and H(O)-6′–O(S)-2	H(O)-3–O-5′ and H(O)-6′–O(S)-2
3			H(O)2′–O3	H(O)-3–O-5′	H(O)-3–O-5′ and H(O)-2′–O-6
5		H(O)-3–O(S)-2	H(O)3–O(S)2 and H(O)2′–O3	H(O)-3–O-5′ and H(O)-6′–O(S)-2	H(O)-3–O-5′ and H(O)-6′–O(S)-2
7		H(O)-6′–O(S)-6	H(O)6′–O(S)6 and H(O)2′–O3	H(O)-3–O-5′	H(O)-3–O-5′ and H(O)-2′–O-6
9		H(O)-3–O(S)-2 and H(O)-6′–O(S)-6	H(O)3–O(S)2, H(O)6′–O(S)6 and H(O)2′–O3	H(O)-3–O-5′ and H(O)-6′–O(S)-2	H(O)-3–O-5′, H(O)-6′–O(S)-2 and H(O)-3′–O(S)-4′
10	-			H(O)-6′–O-6	
11	H(O)-3′–O(S)-2′			H(O)-6′–O-6	
12				H(O)-6′–O-6	H(O)3′–O(S)4′
13				H(O)-6′–O-6	H(O)6′–O(S)2
14	H(O)3′–O(S)2′			H(O)-6′–O-6	H(O)6′–O(S)2
15			H(O)3′–O(S)4′	H(O)-6′–O-6	H(O)3′–O(S)4′

Table 5
Effects of sulfation on the energy differences (kcal/mol) between minima in BA and AB maps

Effect	BA maps				AB maps [a,g]
	E_B-E_A	E_C-E_A	E_D-E_A	E_C-E_B	E_B-E_A
For β-D-Galp-(1→4)-α-D-Galp					
On 2 of β-Gal	+1.0	−0.3	+2.0	−0.7	−1.7
On 4 of β-Gal	0.0	−0.2	−0.3	−0.2	+2.1
β-2-sulfated					
On 2 of α-Gal	+1.8 [b]	+1.9	−0.1	+0.1 [e]	−0.4
On 6 of α-Gal	−0.7 [b]	+0.9 [c]	+1.2	+0.5 [e]	−1.3
β-4-sulfated					
On 2 of α-Gal	−0.2	−0.1 [d]	+0.6 [e]	+1.1	−0.8 [f]
On 6 of α-Gal	+0.8	+1.0	+1.5 [e]	+0.2	+0.5 [f]
For β-D-Galp-(1→4)-3,6-An-α-D-Galp					
On 2 of β-Gal	+0.4	+0.5	+0.3	+0.1	−2.8
On 4 of β-Gal	0.0	−0.1	−0.9	−0.1	+0.8
On 2 of 3,6-AnGal	≈+0.1	≈0.0	≈−1.5	≈−0.2	≈0.0/−1.3 [h]

[a] Calculated from data on Ref. 24 (corrected).
[b] An additional of 0.5 should be subtracted if both sulfates on 2- and 6- are present.
[c] An additional of 1.0 should be subtracted if both sulfates on 2- and 6- are present.
[d] An additional of 1.0 should be added if both sulfates on 2- and 6- are present.
[e] An additional of 0.4 should be subtracted if both sulfates on 2- and 6- are present.
[f] An additional of 0.5 should be added if both sulfates on 2- and 6- are present.
[g] Calculated from data on Ref. 21.
[h] The −1.3 value applies to compounds sulfated at position 4.

calculated strain energy is 0.8 kcal/mol above that of minimum **A**. Free-energy calculations shorten this difference to 0.6 kcal/mol. Fiber diffraction analysis of ι- and κ-carrageenan made about 30 years ago[10–12] indicate ordered conformations with ϕ,ψ angles at −87°,81° (adjusted to −87°,94° in a further refinement[13]) and −97°,108°, respectively. Recent studies[13,14] on the sodium and calcium salts of ι-carrageenan confirmed the previous helical models,[10] yielding ϕ,ψ angles of −89°,109° (sodium salt[13]) and −91°,104° (calcium salt[14]). These geometries also correspond to the **B** region (Fig. 5), which is not the one detected preferentially by MM3, although with this force-field, the **B** minimum is very close in energy with the global one. It should be borne in mind that these geometries correspond to the ordered polysaccharide, where packing forces, inter- and intramolecular bonding can offset weaker forces present in the disaccharide. Moreover, NOE data on the glycoside of compound **12** showed that a conformation similar to that of the **A** minimum prevails in solution.[17] The present work shows that MM3 has detected precisely this region and hydroxymethyl orientation as the minimum-energy one, although not far in energy from the **B** region. The calculated torsional angles for the sulfate groups ($\theta_{H\text{-}n\text{–}C\text{-}n\text{–}O\text{-}n\text{–}S}$) also match those determined by fiber diffraction analysis of ι-carrageenan.[13,14] Force-field calculations have been carried out with compounds **10**, **12** and **15**. The PFOS/MM2 approach for **10** yielded several minima in the **A** region, and a high-energy one (9 kcal above) in the **B** region.[17] The authors have wrongly considered the conformation of the calculated minimum similar to that of the solid state,[16] due to a mistake in the angles convention.[17] The work by Ueda et al.[19] with **10** using a CHARMM-type force-field indicated **A** as the global minimum, very close in energy to those of **B**, **D** and two unidentified regions. The application of molecular dynamics with the same force-field led to a conformation in an unidentified region (Fig. 5) most of the time, in order to keep a helical structure.[19] The rigid-residue analysis of Urbani et al.[18] for **12** showed more flexibility around the **A**–**B** region than for the α linkage. Although no numeric data is available, the maps show **A** as the global minimum, followed closely by **D** and **B**.[18] Le Questel et al.[8] analyzed **12** and **15** using the Tripos force-field. Their maps showed a sharply different shape of the minimum energy region, which just extends "horizontally", probably as a result of an incomplete consideration of the exo-anomeric effect. For **12**, five minima appear within 1 kcal/mol: three are close to region **D** (one of them, the global one, Fig. 5), while the other two appear not far from regions **A** and **B**.[8] For **15**, a similar map is

observed, although the **A**-like minimum becomes the global one. As occurred with neocarrabiose and derivatives,[21] MM3 predicts an energy probably closer to reality for those conformers. It was previously addressed that the β linkage of carrageenans is more difficult to model than the α linkage, due to the larger number of low-lying conformations potentially accessible.[45] The proximity in energy between the **A** and **B** regions in both cases makes it impossible to ascertain that either is predominant by just using computer modeling. Subtle factors in the force-field parameterization may lead to wrong conclusions if these small energy differences are intended to make conclusive.

The strong influence of the sulfation on positions 2 and 4 of the β-D-galactose unit on the conformational features of the α linkage[21,24] was used to explain the strong chemical shift displacements of the ^{13}C NMR signals corresponding to C-1 of the 3,6-anhydro-α-D-galactose unit of a ι-carrageenan with respect to that of an alkali treated λ-carrageenan,[46] as well as that of the C-1 of an α-D-galactose unit of ν-carrageenan with respect to λ-carrageenan.[46,47] This was rationalized on grounds of their main minima being in different regions.[24] This work shows that the β-glycosidic linkage suffers less conformational influence by sulfation of the α-unit, as the calculated minimum remains the same, notwithstanding the sulfation pattern. As expected, the ^{13}C NMR signals corresponding to C-1 of the β-D-galactose units of different carrageenans experience almost no effect by the sulfation pattern of the α-D-galactose units.[48]

Acknowledgements

The author is a Research Member of the National Research Council of Argentina (CONICET). This work was supported by grants from UBA (X087) and CONICET. The author is indebted to Dr A.S. Cerezo for helpful discussions, and to the reviewers of previous manuscripts on the field for their suggestions and hints to improve our work. The last-minute information provided by Dr S. Janaswamy is also acknowledged.

References

1. Stortz, C. A.; Cerezo, A. S. *Curr. Top. Phytochem.* **2000**, *4*, 121–134.
2. Ragazzi, M.; Ferro, D.; Provasoli, A. *J. Comput. Chem.* **1986**, *7*, 105–112.
3. Ferro, D. R.; Pumilia, P.; Cassinari, A.; Ragazzi, M. *Int. J. Biol. Macromol.* **1995**, *17*, 131–136.
4. Huige, C. J. M.; Altona, C. *J. Comput. Chem.* **1995**, *16*, 56–79.
5. Iorizzi, M.; Bifulco, G.; de Riccardis, F.; Minale, L.; Riccio, R.; Zollo, F. *J. Nat. Prod.* **1995**, *58*, 10–26.
6. Ferro, D. R.; Pumilia, P.; Ragazzi, M. *J. Comput. Chem.* **1997**, *18*, 351–367.
7. Lamba, D.; Glover, S.; Mackie, W.; Rashid, A.; Sheldrick, B.; Pérez, S. *Glycobiology* **1994**, *4*, 151–163.
8. Le Questel, J.-Y.; Cros, S.; Mackie, W.; Pérez, S. *Int. J. Biol. Macromol.* **1995**, *17*, 161–175.
9. Anderson, N. S.; Campbell, J. W.; Harding, M. M.; Rees, D. A.; Samuel, J. W. B. *J. Mol. Biol.* **1969**, *45*, 85–99.
10. Arnott, S.; Scott, W. E.; Rees, D. A.; McNab, C. G. A. *J. Mol. Biol.* **1974**, *90*, 253–267.
11. Millane, R. P.; Chandrasekaran, R.; Arnott, S.; Dea, I. C. M. *Carbohydr. Res.* **1989**, *182*, 1–17.
12. Millane, R. P.; Nzewi, E. U.; Arnott, S. Molecular Structures of Carrageenans as Determined by X-ray Fiber Diffraction. In *Frontiers in Carbohydrate Research*; Millane, R. P.; BeMiller, J. N.; Chandrasekaran, R., Eds.; Elsevier: London, 1989; pp 104–131.
13. Janaswamy, S.; Chandrasekaran, R. *Carbohydr. Res.* **2001**, *335*, 181–194.
14. Janaswamy, S.; Chandrasekaran, R. *Carbohydr. Res.* **2002**, *337*, 523–535.
15. Lamba, D.; Segre, A. L.; Glover, S.; Mackie, W.; Sheldrick, B.; Pérez, S. *Carbohydr. Res.* **1990**, *208*, 215–230.
16. Lamba, D.; Burden, C.; Mackie, W.; Sheldrick, B. *Carbohydr. Res.* **1986**, *155*, 11–17.
17. Parra, E.; Caro, H.-N.; Jiménez-Barbero, J.; Martín-Lomas, M.; Bernabé, M. *Carbohydr. Res.* **1990**, *208*, 83–92.
18. Urbani, R.; Di Blas, A.; Cesàro, A. *Int. J. Biol. Macromol.* **1993**, *15*, 24–29.
19. Ueda, K.; Ochiai, H.; Imamura, A.; Nakagawa, S. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 95–106.
20. Ueda, K.; Brady, J. W. *Biopolymers* **1996**, *38*, 461–469.
21. Stortz, C. A.; Cerezo, A. S. *J. Carbohydr. Chem.* **2000**, *19*, 1115–1130.
22. Stortz, C. A.; Cerezo, A. S. *J. Carbohydr. Chem.* **1994**, *13*, 235–247.
23. Stortz, C. A.; Cerezo, A. S. *An. Assoc. Quim. Argent.* **1995**, *83*, 171–181.
24. Stortz, C. A.; Cerezo, A. S. J. *Carbohydr. Chem.* **1998**, *17*, 1405–1419.
25. Stortz, C. A. *Carbohydr. Res.* **1999**, *322*, 77–86.
26. Allinger, N. L.; Yuh, Y. H.; Lii, J.-H. *J. Am. Chem. Soc.* **1989**, *111*, 8551–8566.
27. Allinger, N. L.; Rahman, M.; Lii, J.-H. *J. Am. Chem. Soc.* **1990**, *112*, 8293–8307.
28. Koča, J.; Pérez, S.; Imberty, A. *J. Comput. Chem.* **1995**, *16*, 296–310.
29. Dowd, M. K.; Zeng, J.; French, A. D.; Reilly, P. J. *Carbohydr. Res.* **1992**, *230*, 223–244.
30. Dowd, M. K.; French, A. D.; Reilly, P. J. *Carbohydr. Res.* **1992**, *233*, 15–34.
31. Dowd, M. K.; Reilly, P. J.; French, A. D. *J. Comput. Chem.* **1992**, *13*, 102–114.
32. Dowd, M. K.; French, A. D.; Reilly, P. J. *J. Carbohydr. Chem.* **1995**, *14*, 589–600.
33. MM3 (96). *Bull. QCPE* **1997**, *17*, 3.
34. French, A. D.; Kelterer, A.-M.; Johnson, G. P.; Dowd, M. K.; Cramer, C. J. *J. Mol. Graph. Model.* **2000**, *18*, 95–107.
35. Engelsen, S. B.; Koča, J.; Braccini, I.; Hervé du Penhoat, C.; Pérez, S *Carbohydr. Res.* **1995**, *276*, 1–29.
36. Engelsen, S. B.; Rasmussen, K. *J. Carbohydr. Chem.* **1997**, *16*, 773–788.
37. Koča, J. *J. Mol. Struct.* **1993**, *291*, 255–269.
38. French, A. D.; Kelterer, A.-M.; Johnson, G. P.; Dowd, M. K.; Cramer, C. J. *J. Comput. Chem.* **2001**, *22*, 65–78.

39. Mikros, E.; Labrinidis, E.; Pérez, S. *J. Carbohydr. Chem.* **2000**, *19*, 1319–1349.
40. Stortz, C. A.; Cerezo, A. S. *J. Carbohydr. Chem.*, in press.
41. Rees, D. A. *Polysaccharide Shapes*; Chapman & Hall: London, 1977; p 51.
42. Engelsen, S. B.; Rasmussen, K. *J. Carbohydr. Chem.* **1997**, *16*, 773–788.
43. Barrows, S. E.; Dulles, F. J.; Cramer, C. J.; French, A. D.; Truhlar, D. G. *Carbohydr. Res.* **1995**, *276*, 219–251.
44. Bayley, S. T. *Biochim. Biophys. Acta* **1955**, 194–205.
45. Schafer, S. E.; Stevens, E. S. *Carbohydr. Polym.* **1996**, *31*, 19–22.
46. Falshaw, R.; Furneaux, R. *Carbohydr. Res.* **1994**, *252*, 171–182.
47. Stortz, C. A.; Bacon, B. E.; Cherniak, R.; Cerezo, A. S. *Carbohydr. Res.* **1994**, *261*, 317–326.
48. Stortz, C. A.; Cerezo, A. S. *Carbohydr. Polym.* **1992**, *18*, 237–242.