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Conformational study of digalacturonic acid and sodium digalacturonate in solution

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Abstract

The solution conformation of digalacturonic acid and its sodium salt have been analyzed using nuclear magnetic resonance data and molecular mechanics calculations. The flexibility around the glycosidic linkage was characterized by calculation of the relaxed (Φ,Ψ) potential surfaces for the isolated molecule, and also for dimethyl sulfoxide and aqueous solutions using the CHARMM and SOLVOL programs. The one-bond and three-bond proton–carbon couplings were measured and H-1′–H-4 distances were estimated from NOESY experiments. The calculated potential surfaces were used to determine theoretical ensemble averages of NMR data. The agreement between the experimental and theoretical data is very satisfactory. The calculations show a strong effect of solvent on the solution behavior of both compounds. The vacuum lowest energy conformer of digalacturonic acid is stabilized by solvation, while for sodium digalacturonate the solvent induces a conformational change. An extrapolation of the stable conformers to polysaccharide chains implies that poly(galacturonic acid) occurs in solution as a three-fold helix and sodium poly(galacturonate) as a two-fold helix.

Key words: Conformations; Molecular modelling; Effect of solvent; NMR; Pectin fragments; Digalacturonic acid; Sodium digalacturonate

1. Introduction

Pectins mainly α-(1 → 4)-linked D-galacturonan, are present as structural polysaccharides in the middle lamella and the primary cell wall of higher plants.

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They are part of the diet of man and responsible for many technology and quality aspects in the processing of fruits and vegetables. Uses of pectins as gelling agents in food and some newer uses as stabilizers of acid milk products or as clarifying and decontamination agents are also well known.

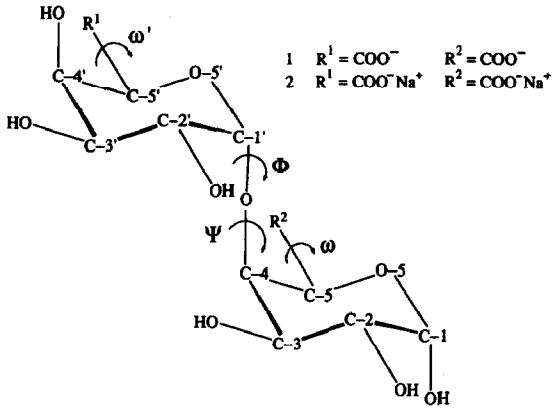
The polyelectrolyte character of the chains, in the acidic form, along with the conformational transitions induced by monovalent and divalent ions, make their structural analysis difficult. Recently, two reports concerning the behavior in aqueous solution of α-(1 → 4)-linked D-galacturono-di- and -tri-saccharides [1] and the methylated pectic disaccharide [2] were described using NMR spectroscopy and molecular modelling. In the first, it was suggested that a right-handed three-fold helical arrangement could be formed by pectic acid oligosaccharides. In the second, when stable conformations found for the methylated pectic disaccharide were extrapolated to a regular polymer structure, right- and left-handed two-fold helices were generated. In each case, it was pointed out that neighboring ions, solvent, or other macromolecules could easily induce a conformational change.

In our laboratory, the influence of the chemical structure of groups at C-5 on the conformational properties of an α-(1 → 4)-glycosidic linkage in solution was studied both experimentally and theoretically. In particular, eight derivatives of O-α-D-galactopyranosyl-(1 → 4)-D-galactopyranose (galabiose) were studied using molecular mechanics calculations. The influence of substituents at positions C-5 and C-5′ on the energy surfaces for hydroxymethyl, carboxylic acid, carboxylate, and sodium carboxylate was investigated and relaxed (Φ,Ψ) maps in vacuum were calculated [3].

The present paper reports the results obtained for O-(α-D-galactopyranosyluronic acid)-(1 → 4)-α-D-galactopyranuronic acid (digalacturonic acid, **1**) and O-(sodium α-D-galactopyranosyluronate)-(1 → 4)-sodium α-D-galactopyranuronate (sodium digalacturonate, **2**) in dimethyl sulfoxide and water using NMR data and molecular mechanics calculations.

2. Experimental

Samples.—The disaccharides O-(α-D-galactopyranosyluronic acid)-(1 → 4)-α-D-galactopyranuronic acid (**1**) and O-(sodium α-D-galactopyranosyluronate)-(1 → 4)-sodium α-D-galactopyranuronate (**2**) were prepared by enzymatic hydrolysis of sodium poly(galacturonate) [4]. The polysaccharide was dissolved in 0.2 M acetate buffer (pH 4.2) and incubated at 30°C with endogalacturonanase (EC 3.2.1.15) for 1 h. The solution was heated at 100°C to denature the enzyme. The oligogalacturonates produced were separated and purified by chromatography on Bio-Gel P-6 using $NaNO_3$ as the eluent (+0.02% NaN_3). Fractions of dp 2 were desalted by ultrafiltration and freeze-dried. Part of the product obtained was diluted in water and passed through a cation-exchange column (IRN 77 resin, H^+). The digalacturonic acid obtained was freeze-dried.



Nomenclature.—The recommendations and symbols proposed by the IUPAC-IUB commission [5] were followed. A schematic representation of the disaccharides and the numbering of atoms is shown in Scheme 1. The relative orientation of the monosaccharide residues about the glycosidic linkage is based on the values of two torsional angles Φ(O-5′–C-1′–O-4–C-4) and Ψ(C-1′–O-4–C-4–C-3). For calculations of the C–H coupling constants, the torsional angles Φ^H(H-1′–C-1′–O-4–C-4) and Ψ^H(C-1′–O-4–C-4–H-4) with reference to the hydrogen atoms were used. The orientation of the acidic groups is defined by the torsional angles ω(O-5–C-5–C-6–O-6) and ω'(O-5′–C-5′–C-6′–O-6′). Interglycosidic proton–proton distances are described as r_{ij}; thus $r_{1'4}$ refers to the distance between H-1′ and H-4.

NMR study.—NMR spectra were obtained on either an AC 300, AM 300, or an AM 400 Bruker spectrometer, all equipped with a process controller, an Aspect 3000 computer, and a variable temperature system. Samples of digalacturonic acid and sodium digalacturonate were dissolved in Me_2SO-d_6 and high quality deuteriated water, respectively (1% solution for ^1H, 2% solution for ^{13}C).

^1H and ^{13}C assignments were made from 2D-COSY-DQF [6] and 2D ^{13}C–^1H correlation [7] spectroscopy, respectively.

COSY spectra were recorded using a 2048 × 512 data matrix and processed with zero-filling in the F1 dimension. The spectral width was 1000 Hz in both dimensions. A sine-bell window function was used prior to Fourier transformation. The ^1H–^{13}C shift correlation spectra were recorded using a 2048 × 256 data matrix and were zero filled in the F1 dimensions. Spectral widths were 5000 Hz in F2 and 1000 Hz in F1. Gaussian multiplication was performed prior to Fourier transformation.

Selective 2D-*J* heteronuclear experiments [8,9] were run to measure $^3J_{C,H}$ coupling constants. To minimize pulse imperfection, EXORCYCLE phase cycling was used for ^{13}C pulses. The ^1H 180 pulse was created using a DANTE sequence [10]. 4K Data points were used in the F2 dimension. After Fourier transformation in the F2 dimension, only the slices corresponding to ^{13}C lines of interest were treated in the F1 dimension in which no apodization function was used.

One-bond coupling constants were measured using DEPT experiments with polarization transfer from ^{1}H to ^{13}C nuclei for refocused ^{1}H-coupled spectra.

Phase-sensitive NOESY [11] was acquired with several mixing times from 0.12 to 0.8 s. A 20 ms random delay was introduced to cancel scalar correlation effects. A 512 × 1024 data matrix was used and zero-filled to 1024 × 1024. Prior to Fourier transformation, sine-squared weighting functions were applied in both directions. NOESY cross-peak intensities were evaluated by volume integration after symmetrization of the matrix.

Conformational analysis.—The starting geometry of the disaccharides were derived from the X-ray crystal structure of galabiose [12] as previously described [3]. Replacement of hydroxymethyl groups by carboxylic groups was performed using crystallographic coordinates of acid compounds [13–15].

Energy calculations were performed with the CHARMM program [16] using its force field as interfaced in QUANTA software [17], version 3.3, from Molecular Simulations, Inc. Relaxed maps were prepared by rotating the rigid residues of **1** and **2**, to each combination of Φ and Ψ on a 10° grid. At each of the Φ_i, Ψ_j grid points the energy was minimized by allowing the Cartesian coordinates of each atom to vary. Geometry optimization was carried out with 500 steps of a conjugate gradient procedure in Cartesian coordinates. At each step, potential energy and energy gradients were calculated. Minimization was carried out until a root mean square (rms) value of less than 0.1 kcal/mol.A^2, (1 cal = 4.1868 J) of the calculated gradient was obtained. Then, the orientations of pendent groups were refined. This leads to small differences in relative energies of conformers in vacuum in comparison with our previous results where a such refinement was not carried out.

Solvent-specific relaxed conformational maps were also calculated by means of the SOLVOL program to take into account the influence of the solvent [18].

Statistical mechanics was utilized to calculate ensemble average molecular characteristics from the relaxed map. The relative population of each conformer is given by the Boltzmann distribution such that:

$$P_i = \exp(-\Delta G_i(\Phi,\Psi)/RT)/Q)$$

where Q is the classical partition function. Any molecular parameter M may be calculated for each conformational microstate and the ensemble average parameter $\langle M \rangle$, defining a "virtual" conformation, is given as:

$$\langle M \rangle = \Sigma_i P_i M_i$$

The vicinal heteronuclear and the one-bond C,H coupling constants were calculated by using published Karplus-type relationships [19,20].

Calculations were performed on an IRIS Indigo 2 station; molecular drawings were produced by using the QUANTA software. Isoenergy contour lines were generated using a home-made program CARTO.

3. Results and discussion

NMR data.—The proton spectra of digalacturonic acid **1** in Me_2SO-d_6 and sodium digalacturonate **2** in deuteriated water are shown in Fig. 1. Both ^{1}H and

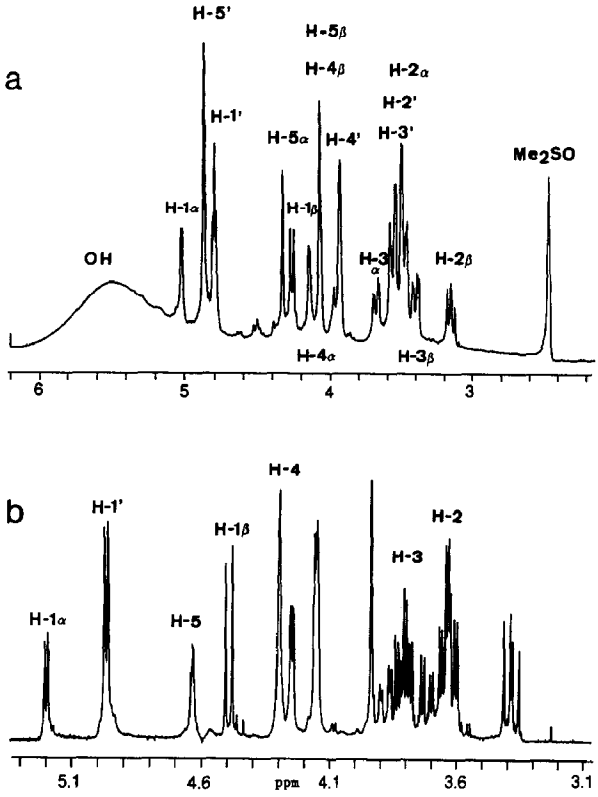


Fig. 1. Proton spectra of (a) digalacturonic acid **1** in Me_2SO-d_6 at 30° and (b) sodium digalacturonate **2** in D_2O at 30°C.

^{13}C chemical shifts were assigned by means of COSY and $^{1}H-^{13}C$ shift correlation experiments. The chemical shift values of the protons and carbons involved in the glycosidic linkage for both disaccharides are reported in Tables 1 and 2, with the corresponding heteronuclear coupling constants through one and three bonds. For **2**, several signals are sensitive to the anomeric configuration, giving slightly different values.

Table 1
^{1}H and ^{13}C chemical shifts [a] (ppm) and heteronuclear coupling constants [a] (Hz) of digalacturonic acid **1** in Me_2SO-d_6 at 30°C

Unit	H-1'	H-4	C-1'	C-4
α Anomer	5.05	4.18	93.22	77.03
reducing				
β Anomer	4.30	4.10	97.75	76.56
nonreducing	4.82	3.96	99.45	70.92
	$^{1}J_{C\text{-}1',H\text{-}1'}$	$^{1}J_{C\text{-}4,H\text{-}4}$	$^{3}J_{H\text{-}1',C\text{-}4}$	$^{3}J_{C\text{-}1',H\text{-}4}$
168.8	148		3.6	4.85

[a] Only values related to atoms involved in the glycosidic linkage are reported.

Table 2
^{1}H and ^{13}C chemical shifts [a] (ppm) and heteronuclear coupling constants [a] (Hz) of sodium digalacturonate **2** in deuteriated water at 30°C

Unit	H-1′		H-4		C-1′		C-4	
α Anomer reducing	5.19		4.30		93.65		79.64	
β Anomer nonreducing	4.25		4.25		97.58		78.64	
	4.95		4.15		α 100.50		72.26	
					β 100.64			
	$^{1}J_{C\text{-}1',H\text{-}1'}$		$^{1}J_{C\text{-}4,H\text{-}4}$		$^{3}J_{H\text{-}1',C\text{-}4}$		$^{3}J_{C\text{-}1',H\text{-}4}$	
	α	β	α	β	α	β	α	β
	171.55	172.5	149.35	148.9	3.35	3.8	5.9	5.15

[a] Only values related to atoms involved in the glycosidic linkage are reported.

The $^{3}J_{C,H}$ values have been shown to depend among several parameters on the dihedral angle for the C–O–C–H segment via a Karplus-type relationship. This relation was established [19] from data obtained with for a series of rigid carbohydrates having known X-ray structures. Here, the corresponding dihedral angles represent the glycosidic torsional angles:

$$\Phi^{H} = \text{C-4–O-4–C-1′–H-1′}$$

$$\Psi^{H} = \text{C-1′–O-4–C-4–H-4}$$

Also the angular dependence of $^{1}J_{C,H}$ on these glycosidic torsional angles in α-linked oligosaccharides was described [20] and characterized in the general form:

$$^{1}J_{C,H} = A \cos 2\theta + B \cos \theta + C \sin 2\theta + D \sin \theta + E$$

with different values of the constants A–E for the α and β anomers and for $^{1}J_{C,H}$ involving either the anomeric centre or corresponding to the aglycon C–H bond.

However, like other NMR parameters, the observed coupling constant values represent average values, because of all the conformations accessible by the molecule on the NMR timescale. This means that interpretation of experimental data as such, without the help of molecular modeling, leads only to a "virtual" conformation with no real physical meaning. However, methods exist to generate more realistic representations, i.e., the ensemble of structures consistent with the experimental data, also taking into account considerations concerning the frequency with which transitions between the different structures occur [21]. Then, calculations using computed simulation and methods based on potential energy functions yields the ensemble of available structures from which the NMR data may be interpreted adequately, particularly if the solvent effects are taken into account.

Spatial proximities were estimated through phase-sensitive NOESY experiments using several mixing times in order to establish the NOE build-up of several correlated protons (H-1′ and H-4; H-3′ and H-5′). In the isolated spin-pair approximation [22] (ISPA) and at zero mixing time, the intensities are inversely proportional to the sixth power of the distance between the correlated protons.

The dependence on correlation time can be eliminated from the calculation by scaling all the distances with respect to a known reference distance which is assumed to have the same correlation time as the proton–proton pair of interest. The following equation is typical of such a scaling:

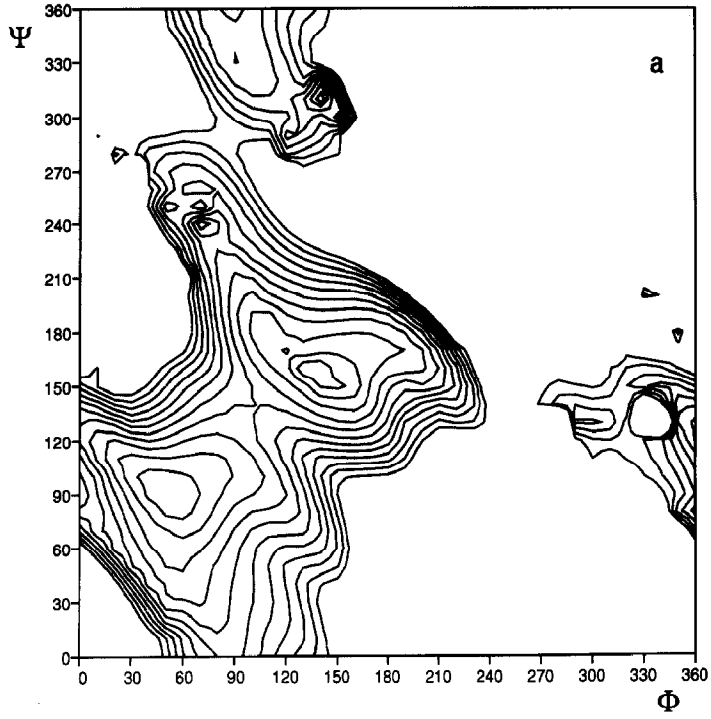
$$r_{ij} = (\sigma_{ref}/\sigma_{ij})^{1/6} r_{ref}$$

where σ_{ref} and r_{ref} are the cross-relaxation rate and the separation of the reference pair of protons; σ_{ref} and σ_{ij} were determined from the initial NOE build-up rates after relative volume integration of cross- and diagonal-peaks. Here as reference distance, we used H-3′–H-5′ = 2.59 Å for **1** and H-3′–H-5′ = 2.54 Å for **2** as determined from calculated structures. Then, the corresponding virtual H-1′–H-4 distance was estimated as 2.88 Å for the acid **1** and 2.55 Å for the sodium salt **2**.

Molecular modeling.—The relaxed conformational (Φ,Ψ) potential energy surfaces for digalacturonic acid and sodium digalacturonate in the isolated state have already been presented in a previous study [3]. The existence of six low-energy areas, as well as the total accessible space covered by the 12 kcal/mol contour line, suggest conformational flexibility for digalacturonic acid. On the other hand, the total accessible space of the sodium digalacturonate map is smaller and only two stable conformers can be located, implying lower conformational flexibility for this compound.

The solvent specific relaxed (Φ,Ψ) potential surfaces for digalacturonic acid **1** in dimethyl sulfoxide and in water are shown in Fig. 2, together with the map computed for the isolated state. The main features of the vacuum map, such as the general shape delimited by the 12 kcal/mol contour and the number and position of the low energy areas are preserved on the two solvent maps. The principal differences consist in a different stabilization of the minima and in the change of the energy barrier between them. The two lowest energy areas are observed at Φ,Ψ values of (150°,150°) and (50°,90°). These areas are comparable in relative energy and in size on the vacuum map but show substantial differences in the two others maps.

From the six minima (CC1–CC6) localized on the maps, the relative energy of two, namely CC5 at (90°, − 30°) and CC6 at (− 60°,130°) are too high and these minima are not involved in the equilibrium mixture. Relative energies and Boltzmann abundances of the four lowest minima are listed in Table 3. Solvation does not change the order of the minima but increases the stabilization of the lowest minimum CC1, the CC2 minimum having a relative energy of 1.8 kcal/mol in Me_2SO and 3.2 kcal/mol in water in comparison to 0.5 kcal/mol in the isolated state. Thus, the population of conformer CC1 increases from 69.7% in vacuum to 99.5% in water. An analysis of the solvation contributions revealed that the stabilization of this conformer is due to the electrostatic term. A large value of 7.66 D of the dipole moment of CC1 conformer is responsible for the enhanced stabilization of this conformer by intermolecular dipolar interactions with increasing polarity of solvent. In contrast to conformer CC1, the population of CC2 with a dipole moment of 5.34 D decreases with increasing polarity of the solvent. These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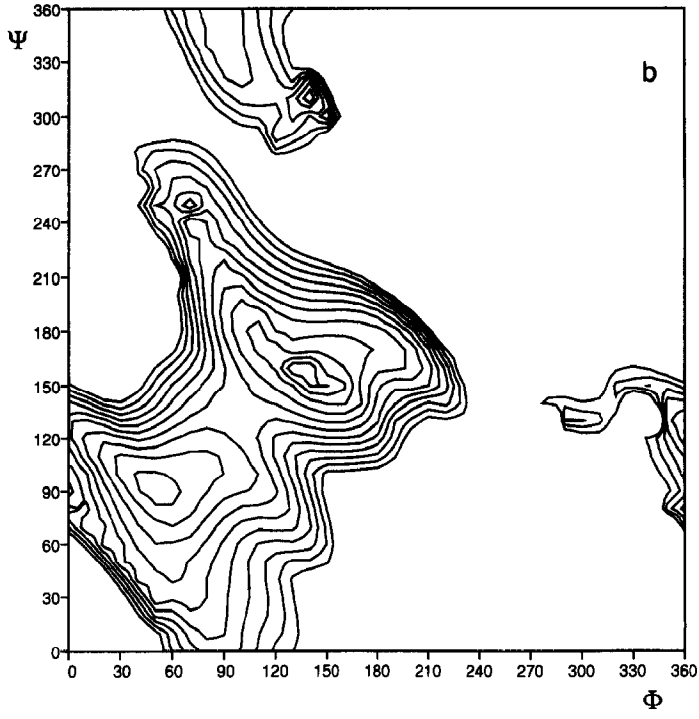
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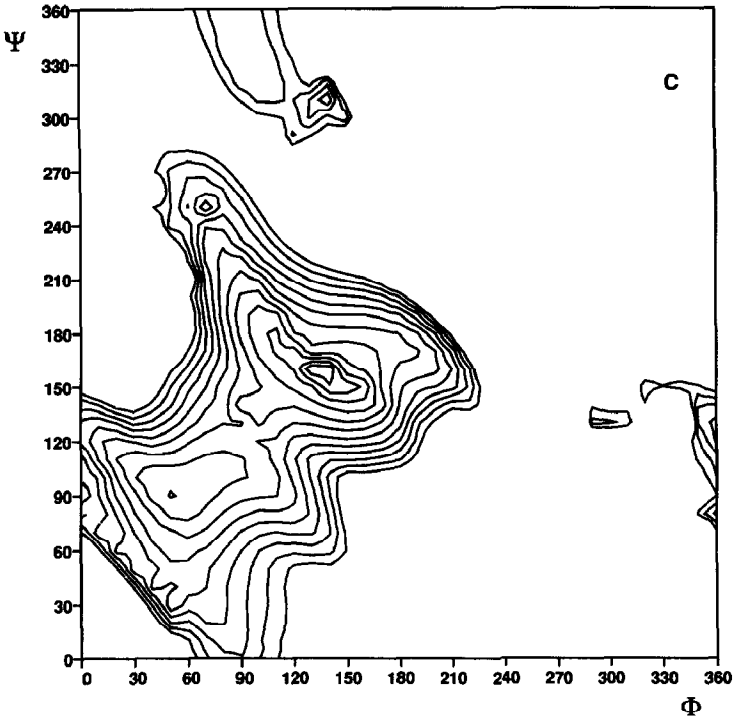


Fig. 2. Relaxed potential energy surface of digalacturonic acid **1** in (a) the isolated state, (b) dimethyl sulfoxide, and (c) water as a function of the Φ and Ψ torsional angles. Isoenergy contours are drawn up to 12 kcal/mol above the energy minimum.

two conformers are present almost exclusively in an equilibrium mixture, the minima of CC3 and CC4 having negligible abundances.

In spite of the dominance of two conformers in an equilibrium mixture based on the relative energies of four conformers, digalacturonic acid exhibits quite a large libration motion. This might be expected from the shape of the two lowest energy

Table 3
Relative energy ΔE and calculated molar fractions of the four minima (CC1–CC4) of digalacturonic acid **1** and the two minima (N1 and N2) of sodium digalacturonate **2** in vacuum and in solution

	ΔE (kcal mol^{-1})			Molar fraction (%)		
	Vacuum	Me_2SO	Water	Vacuum	Me_2SO	Water
CC1	0.0	0.0	0.0	69.7	94.9	99.5
CC2	0.5	1.8	3.2	30.2	5.1	0.5
CC3	4.2	6.1	8.1	0.1	0.0	0.0
CC4	4.2	6.0	8.2	0.1	0.0	0.0
N1	0.0	18.0	39.8	99.6	0.0	0.0
N2	3.3	0.0	0.0	0.4	100.0	100.0

Table 4
Selected internal geometry parameters (°) and hydrogen bond pattern of the two minima of digalacturonic acid **1** and the sodium digalacturonate **2**

	CC1	CC2	N1	N2
Φ	150	50	100	160
Ψ	150	90	150	120
Φ^H	32	−72	−20	41
Ψ^H	29	−35	29	−10
ω'	−108	−110	−87	−65
ω	61	69	−61	−32
H bond	O-2′(H)···O-6		O-5′···(H)O-3 O-2′(H)···O-6	
r(O··H)	1.8		1.97 1.99	
r(O··O)	2.74		2.85 2.87	

regions where several conformers are encompassed by the 1 kcal/mol contour. Then, if one considers not only the four conformers mentioned above, but the whole map, the libration motion about Φ and Ψ will lead to different molar fractions. Thus, using this approach, the population of the CC1 conformer is 20.6, 52.7, and 68.8% in vacuum, dimethyl sulfoxide, and water, respectively. Similarly, for the CC2 conformer the calculated population is 8.8, 2.7, and 0.4%, respectively. The comparison of these two sets of populations clearly shows that digalacturonic acid undergoes libration motion with Φ and Ψ fluctuating about 20° from the local minima. Also, these results indicate that for the calculation of the average values corresponding to observable parameters, it is necessary to take into account the average over the whole map, not only over the local minima.

Selected geometrical parameters of the two lowest energy minima are listed in Table 4 and molecular drawings are presented in Fig. 3. The deepest minimum CC1 appears at an Φ value of 150°. Thus, the C-4 atom is in an antiperiplanar position relative to the ring oxygen of the nonreducing residue. This orientation corresponds to the second lowest minimum of the torsional potential of the exocyclic C–O bond in the axial configuration [18]. It is likely that the electrostatic interactions of carboxyl groups with the ring oxygen decrease the influence of the exo-anomeric effect. Also this conformation is stabilized by interresidue hydrogen bonding involving O-2′(H) and O-6, with an oxygen-to-oxygen distance of 2.74 Å which further contributes to the stabilization of this conformation. The second minimum CC2 is at a Φ value of 50°. In this case, C-4 atom is synclinal relative to O-5′. This orientation corresponds to the main minimum for the rotation about the glycosidic linkage. In both conformations, the torsion angle ω' describing the orientation of the carboxylic group of the nonreducing unit is around −110°. This conformation is stabilized by an intraresidue hydrogen bond created between the acid group and the axial O-4′ that influences this unusual orientation. In the

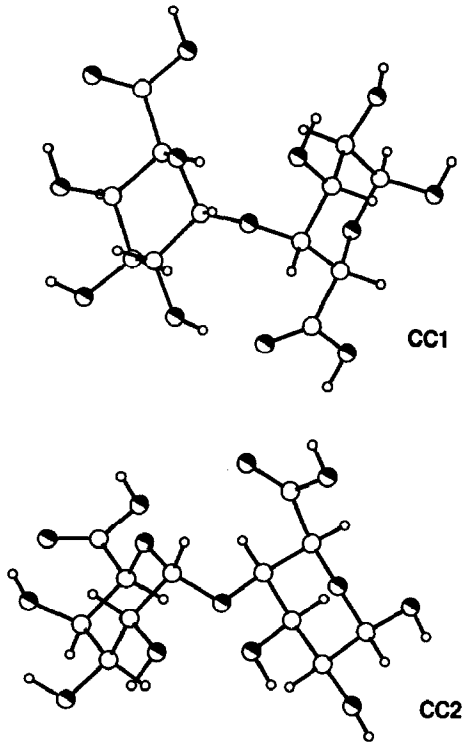
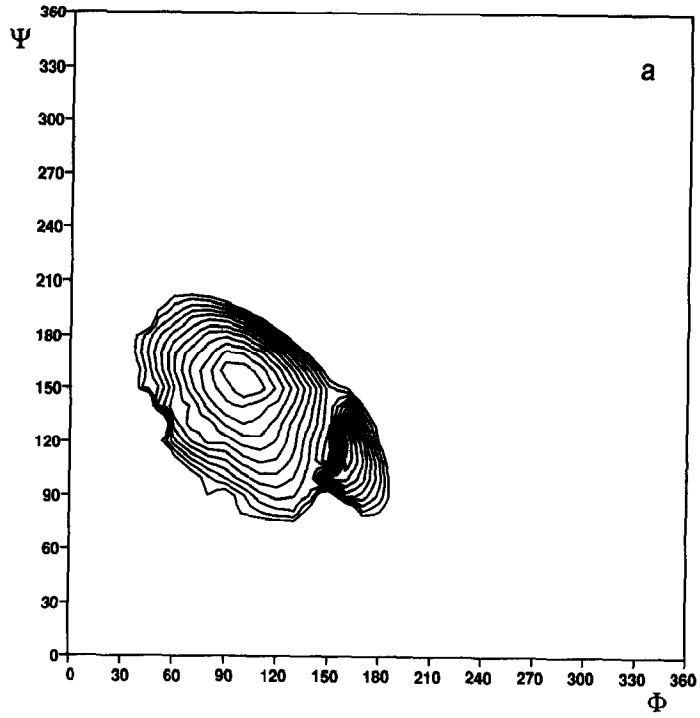


Fig. 3. Representation of the stable conformers of digalacturonic acid **1**.

reducing unit, the ring oxygen atom and the carboxylic oxygen are in an approximately antiperiplanar orientation.

The computed potential surfaces for sodium digalacturonate **2** are shown in Fig. 4 and molecular drawings are presented in Fig. 5. The solvent-specific relaxed maps show different shapes as compared to the corresponding vacuum map. In the isolated state, two minima can be located at Φ,Ψ values of (100°,150°) for N1 and (160°,120°) for N2. The lowest energy conformer is N1, the minimum N2 being 3.3 kcal/mol higher in relative energy (Table 3). Evaluation of solvation energies at each grid point of the map leads to a strong stabilization of the area centred around N2 and disappearance of the accessible space around N1. Thus, solvation reduces the conformational freedom and induces conformational change for this compound. The delicate balance of the intra- and inter-molecular electrostatic interactions plays a crucial role in the conformational properties of **2**.

In vacuum, the strong repulsive electrostatic interactions between charged groups and between counter ions are responsible for the preference of the extended conformation N1. In this conformation the charges are on opposite sites of the molecule and well separated. The conformation N1 is also stabilized by two interresidue hydrogen bonds between O-5′–O-3 and O-2′–O-6 with oxygen-to-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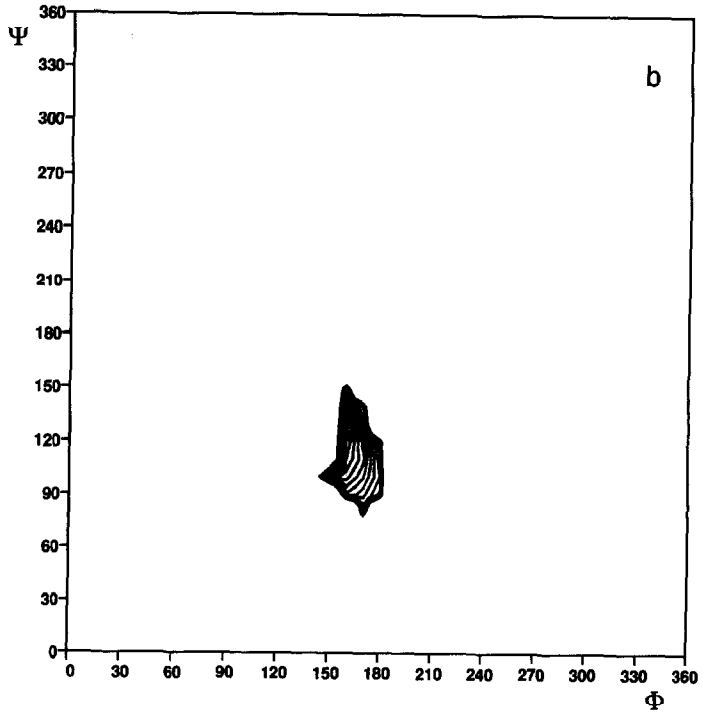
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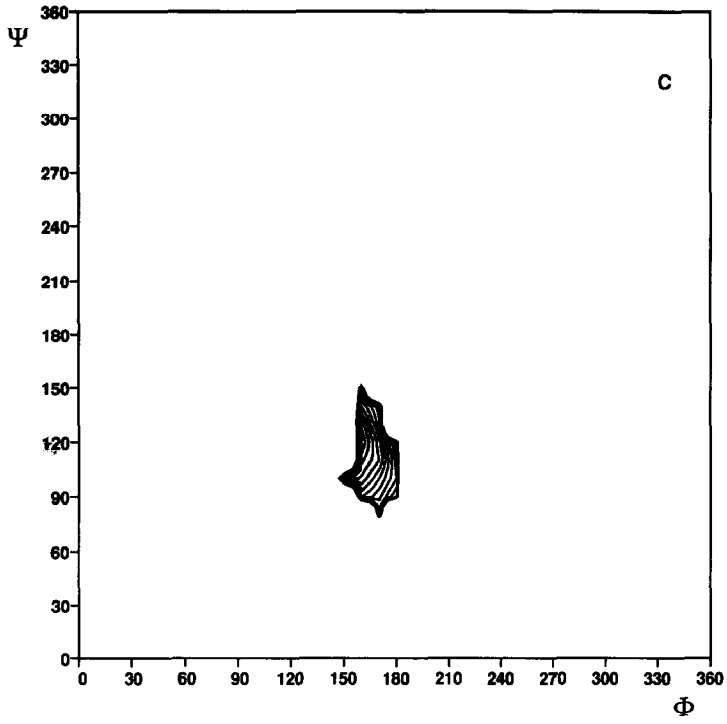

Fig. 4. Relaxed potential energy surface of sodium digalacturonate **2** in (a) the isolated state, (b) dimethyl sulfoxide, and (c) water as a function of the Φ and Ψ torsional angles. Isoenergy contours are drawn up to 12 kcal/mol above the energy minimum.

oxygen distances of 2.85 and 2.87 Å, respectively (Table 4). In polar solvents, this strong intramolecular electrostatic repulsion is diminished by the polarity of the solvent. On the other hand, conformer N2 is stabilized by intermolecular electrostatic interactions due to a large dipole moment of 23.5 D and, in polar solvents, this conformer is the dominant one (Table 3). The C-4 atom is in the anticlinal position relative to O-5′. The exo-anomeric effect, which usually favours the synclinal orientation of C-4 is, again, counter-balanced by electrostatic interactions which, in the case of conformer N2, favor an antiperiplanar orientation of the C-4 atom. As previously in the case of **1**, a libration motion takes place and the population calculated from the whole map increases, from 0.2% in vacuum to 75.3% in Me_2SO and to 87.3% in water.

Comparison with solution properties.—The known angular dependence of carbon–proton coupling constants through one [20] and three bonds [19], makes it possible to calculate theoretical $\langle J_{C,H} \rangle$ values and compare them with experimental values (Tables 5 and 6). There is a good agreement between the observed couplings and the ensemble averages computed in Me_2SO for digalacturonic acid **1** and in aqueous solution for sodium digalacturonate **2**. The only small discrepancy is for the one-bond heteronuclear coupling between C-4 and H-4. The

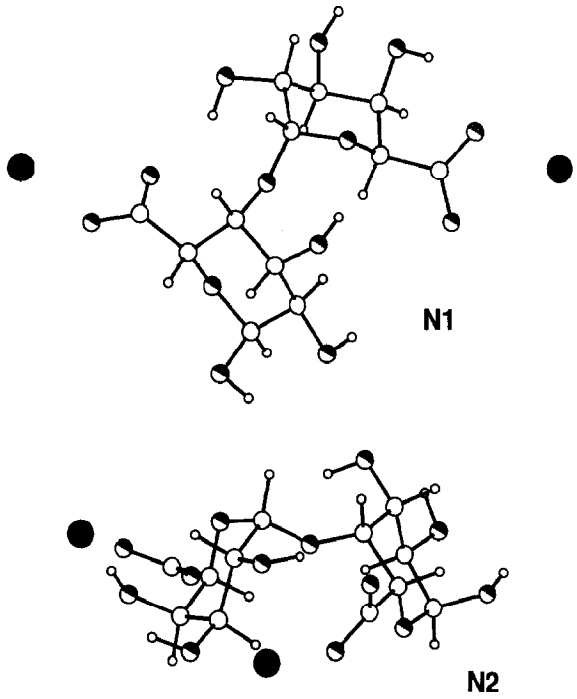

Fig. 5. Representation of the stable conformers of and sodium digalacturonate 2.

measured value of 148 Hz is 2.3 Hz larger than the computed average value of the acid compound and there is a difference of 2.4 Hz for the sodium digalacturonate compound. The abundance of the digalacturonic acid conformers can also be computed in aqueous solution at 25°C. Then a comparison can be made with the experimental data published by Hricovini et al. [1]. The average coupling constant after MM2CARB calculations has led these authors to $\langle {}^3J_\Phi \rangle$ and $\langle {}^3J_\Psi \rangle$ values of 2.3 and 4.0 Hz, respectively. From our calculations, values of 2.73 and 3.93 Hz are

Table 5
Experimental and calculated carbon–proton coupling constants (Hz) and H-1′–H-4 distances of digalacturonic acid 1. Ensemble averages (⟨⟩) were obtained by averaging over the whole map

	$r_{1'4}$	$^1J_{C\text{-}1'\text{-}H\text{-}1'}$	$^1J_{C\text{-}4\text{-}H\text{-}4}$	$^3J_{H\text{-}1'\text{-}C\text{-}4}$	$^3J_{C\text{-}1'\text{-}H\text{-}4}$
CC1	2.32	169.4	146.7	4.08	4.36
CC2	3.03	169.2	146.5	0.88	3.82
CC3	3.33	169.5	149.0	2.14	2.88
CC4	3.64	169.6	150.2	5.20	6.47
⟨Vacuum⟩	2.69	166.4	144.3	2.73	3.93
⟨Me_2SO⟩	2.49	168.2	145.7	3.64	4.21
⟨Water⟩	2.40	169.4	146.7	4.03	4.32
Experimental	2.83	168.8	148.0	3.60	4.85

Table 6
Experimental and calculated carbon–proton coupling constants (Hz) and H-1′–H-4 distance of sodium digalacturonate **2**. Ensemble averages (⟨⟩) were obtained by averaging over the whole map

	$r_{1'4}$	$^1J_{C\text{-}1'\text{-}H\text{-}1'}$	$^1J_{C\text{-}4\text{-}H\text{-}4}$	$^3J_{H\text{-}1'\text{-}C\text{-}4}$	$^3J_{C\text{-}1'\text{-}H\text{-}4}$
N1	2.14	170.0	146.7	4.94	4.34
N2	2.35	169.4	146.9	3.33	5.45
⟨Vacuum⟩	2.19	167.0	144.4	4.76	3.99
⟨Me_2SO⟩	2.35	168.1	145.9	3.35	5.38
⟨Water⟩	2.35	169.4	146.9	3.36	5.43
Experimental	2.55	171.55	149.35	3.35	5.90

obtained for the isolated state. Taking into account the solvent effect, computed values of 4.03 and 4.32 Hz are in close agreement with the experimental data published of 3.7 and 4.9 Hz [1].

A comparison of H-1′–H-4 distances (Tables 5 and 6) shows that the difference between calculated ensemble averages $\langle r_{1'4} \rangle$ and $r_{1'4}$ values estimated from experimental data is less than 15%. Considering the approximations involved in estimating proton–proton distance from NOESY experiments, this agreement is satisfactory.

An extrapolation of stable conformers to regular polysaccharide chains shows that the conformer CC1 corresponds to a left handed helix with n between -2.5 and -3. The conformer CC2 corresponds to a right-handed helix with the number of residues per turn of the helix being $n = 3.8$. This implies that, in solution, a poly(galacturonic) chain is predominantly in the form of three-fold left-handed helix with a small part having a right-handed chirality. The amount of sequences with right-handed chirality is decreasing with increasing polarity of solvent. On the other hand, calculations suggest that the sodium poly(galacturonate) chain adopts a two-fold helical conformation and that the solvent induces a change of the repeating period.

These qualitative models can be compared with experimental data of neutral pectate and pectic acid. Circular dichroism studies [23] of calcium poly(galacturonate) yielded the model of a two-fold helical chain. On the basis of X-ray diffraction of polycrystalline fibres [24], three-fold right-handed helices were proposed for pectic acid and sodium pectate. More recently, the left-handed helix was suggested for poly(galacturonate) chains based on electron microscopy measurements [25]. The results of our calculations are in qualitative agreement with these data and indicate different models for poly(galacturonic) acid and sodium poly(galacturonate). The calculated energy differences suggest that small changes in Φ and Ψ are feasible and can yield other chain structures. However, to develop more realistic solution models for these polysaccharides, a Monte Carlo method must be used. These calculations are in progress in our laboratory.

4. Conclusions

Molecular mechanics calculations of the relaxed conformational energy surfaces for digalacturonic acid **1** and sodium digalacturonate **2** in solution show that these

compounds display quite different solution behaviour. Significant differences are observed between the conformations of **1** and **2** in the isolated state and in solution. Whereas the lowest energy conformer CC1 (150°,150°) of **1** is stabilized by the solvent, for **2**, solvation induces a shift of conformation from N1 (100°,150°) to N2 (160°,120°) which becomes the dominant one.

The extrapolation of the solution behaviour of disaccharides to polysaccharides indicates that the poly(galacturonic acid) may occur in a three-fold helical structure with predominance of left-handed chirality. By contrast, the sodium polygalacturonate chains occur in a two-fold helical structure.

The concordance of calculated and experimental data suggests that the force field with incorporation of the solvent effect used for the calculation of relaxed Φ,Ψ surfaces of digalacturonic acid and sodium digalacturonate correctly describes the solution behaviour of noncharged, as well as charged, compounds.

References

[1] M. Hricovini, S. Bystricky, and A. Malovikova, *Carbohydr. Res.*, 220 (1991) 23–31.
[2] S. Cros, C. Hervé du Penhoat, N. Bouchemal, H. Ohassan, A. Imberty, and S. Pérez, *Int. J. Biol. Macromol.*, 14 (1992) 313–320.
[3] C. Gouvion, K. Mazeau, and I. Tvaroska, in preparation.
[4] J.F. Thibault, *Carbohydr. Polym.*, 3 (1983) 259–272.
[5] IUPAC-IUB Joint Commission on Biochemical Nomenclature, *Eur. J. Biochem.*, 131 (1983) 5–7.
[6] A. Wokaun and R.R. Ernst, *Chem. Phys. Lett.*, 52 (1977) 407–412.
[7] A. Bax, *J. Magn. Reson.*, 53 (1983) 517–520.
[8] A. Bax and R. Freeman, *J. Am. Chem. Soc.*, 104 (1982) 1099–1100.
[9] C. Morat, F.R. Taravel, and M.R. Vignon, *Carbohydr. Res.*, 163 (1987) 265–268.
[10] G. Bodenhausen, R. Freeman, and G.A. Morris, *J. Magn. Reson.*, 23 (1976) 171–175.
[11] G. Bodenhausen, H. Kogler, and R.R. Ernst, *J. Magn. Reson.*, 58 (1984) 370–388.
[12] G. Svensson, J. Albertsson, C. Svensson, G. Magnusson, and J. Dahmen, *Carbohydr. Res.*, 146 (1986) 29–38.
[13] L.J. DeLucas, G.L. Gartland, and C.E. Bugg, *Carbohydr. Res.*, 62 (1978) 213–221.
[14] S. Thanomkul, J.A. Hjortas, and H. Sorum, *Acta Cryst., Sect. B*, 32 (1976) 920–922.
[15] M.D. Newton and G.A. Jeffrey, *J. Am. Chem. Soc.*, 99 (1977) 2413–2421.
[16] B.R. Brooks, R.E. Bruccoleri, B.D. Olafson, D.J. States, S. Swaminathan, M. Karplus, *J. Comput. Chem.*, 4 (1983) 187–217.
[17] Quanta 3.3, Molecular Simulations, Burlington, MA, USA.
[18] I. Tvaroska and T. Kozar, *J. Am. Chem. Soc.*, 102 (1980) 6929–6935.
[19] I. Tvaroska, M. Hricovini, and E. Petrakova, *Carbohydr. Res.*, 189 (1989) 359–362.
[20] I. Tvaroska and F.R. Taravel, *Carbohydr. Res.*, 221 (1991) 83–94.
[21] J.P. Carver, *Curr. Opinion Struct. Biol.*, 1 (1991) 716–720.
[22] A. Kumar, G. Wagner, R.R. Ernst, and K. Wüthrich, *J. Am. Chem. Soc.*, 103 (1981) 3654–3658.
[23] A. Cesaro, A. Ciana, F. Delben, G. Manzini, and S. Paoletti, *Biopolymers*, 21 (1982) 431–449.
[24] M.D. Walkinshaw and S. Arnott, *J. Mol. Biol.*, 153 (1981) 1055–1073.
[25] G.C. Ruben and G.H. Bokelman, in G.W. Bailey (Ed.), *Proceedings of the 45th Annual Meeting of the Electron Microscopy Society of America*, San Francisco Press, San Francisco, CA, 1987, pp 966–967.