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Configurational stability and molecular dynamics of acetal-linked pyruvate substituents in polysaccharides [†]

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Abstract

Pyruvate groups occur naturally in many microbial polysaccharides as nonsaccharidic components and significantly affect their physicochemical and biological properties. The configuration of the acetal carbon of pyruvate groups is mainly influenced by the favoured equatorial orientation of the methyl group. Evaluation of conformational energies has been carried out to assess the relative stabilities of the *R* and *S* isomers as a function of configuration and torsional angles for several residue models, including methyl 4,6-*O*-(1-carboxyethylidene)-α-D-galactopyranoside (**1**). Different levels of theoretical approach are used ranging from *ab initio*, semiempirical (AM1), and molecular mechanics (MM) methods up to molecular dynamics (MD). The higher stability of the isomer *R* of **1** was demonstrated by all of the methods used, thus giving full agreement with the NMR data on the natural compounds.

1. Introduction

Increasing attention is being paid to the possible physiological role of such substituents as acetate and acetal-linked pyruvate but also, less frequently, succinate, formate [1], 3-hydroxybutanoate [2,3], and L-glycerate [4], in the microbial exopolysaccharides [5–7]. Substituents of the exopolysaccharides from *Rhizobia* sp. seem to act as determinants in the symbiotic association between the microorgan-

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isms and the host leguminous plants [5,8]. The presence of pyruvate on exopolysaccharides is one of the common features required by a number of *Rhizobium leguminosarum* biovar strains for successful symbiosis [7], and mutants of *Rhizobium* strains failing to substitute their exopolysaccharides are defective in nodule invasion, one of the steps involved in the symbiotic association [9].

Pyruvate substitution may occur on the terminal sugar unit of the side chain, as in the case of xanthan. Other polysaccharides, such as that from *Rhizobium trifolii* strain TA1, contain two pyruvate groups on the two neighbouring terminal sugars of the side chain, which further enhance the polyelectrolytic character of the polymer [10]. In a few cases the charged pyruvate group occurs in the main chain, as on every second sugar of the repeat unit in galactoglucan [11]. The presence of a pyruvate group dramatically modifies the physicochemical properties of these polysaccharides because, in addition to introducing a new chiral centre at the acetal carbon, it generates a pH-dependent polyelectrolytic character through the distribution of carboxylic groups on the chain, as has been shown for samples of xanthan differing in pyruvate content [12]. Therefore, the perturbation is exerted not only on the conformation of the glycosyl residue at which it is linked, but it may be extended to both the macromolecular conformation and the supramolecular level.

In order to understand the influence of pyruvate on the properties of exopolysaccharides, it is necessary to separate the perturbation arising from the general polyelectrolytic aspects and those introduced by pyruvate at a local molecular level. In a previous study [11] the presence of pyruvate substituents in the galactoglucan repeat unit was found to decrease the accessible conformation space of the disaccharide with a further shift in the population of the conformers. It was concluded that the presence of the substituents not only ensures the solubility of the polysaccharide but also affects the local conformation. However, neither the conformational freedom and preferred orientation of the carboxylic group, nor the stability of the configuration (*R*) of the acetal linked to the galactose unit were ascertained.

A peculiar regularity has been shown by means of NMR spectroscopy for the absolute configuration of the acetal carbon atom of pyruvate linked to O-4 and O-6 of D-glycopyranosyl residues of a number of bacterial polysaccharides. In particular, the *S* configuration is observed when pyruvic acid is linked to D-glucopyranosyl or D-mannopyranosyl residues and the *R* configuration when it is linked to D-galactopyranosyl residues [13,14]. Thus, in all cases the methyl group is disposed equatorially, namely with the same orientation with respect to the 1,3-dioxane ring. This regularity may simply arise from the thermodynamic stability of one of the two enantiomeric forms, without invoking a biological specificity, a consideration that motivated the undertaking of this study. Ascertainment of the thermodynamic stability should provide a useful basis for further investigations on the biosynthetic mechanism and on the synthetic approach to pyruvate substitution.

Research on conformational stability and on the effect of the ionization of the carboxyl group is presented here with model molecules (from the most simple

1,3-dioxane (**2**) model to methyl 4,6-*O*-(1-carboxyethylidene)-α-D-galactopyranoside (**1**), using different theoretical approaches, such as molecular mechanics, *ab initio* and AM1 calculations, and molecular dynamics. The second goal of the present paper is to compare the applicability of these different methods for one particular structural problem, namely the preference of *R* vs. *S* configuration of the pyruvate substituents.

2. Methods

Three levels of sophistication were used to evaluate the total energy of the molecules studied. First, the *ab initio* method was applied within the solution of the Hartree–Foch equation of the molecular system, taking into account all electrons and nuclei. Two basis sets were used namely minimal STO 3G [15] and split-valence 3-21G [16], to study the effect of the basis set on the results of conformational stability. The small basis sets such as 3-21G gives a reasonable trend of stability of the *R* vs. *S* configuration, although for the conformational search along the glycosidic bond 3-21G is not sufficient and the extensive (6-31 + G*) basis set would be necessary [17]. The MONSTERGAUSS program package [18] was explored in this type of calculation. On the semiempirical level of quantum chemical methods, the AM1 parametrization [19] was used, exploring the MOPAC 5.0 package [20]. The reason for using AM1 rests on the fact that it gives satisfactory results for the geometry of carbohydrates [21], although it does not give accurate representation of the energy barriers concerning the glycosidic anomeric centre.

The molecular mechanics (MM) and molecular dynamics (MD) calculations were based on interatomic potentials using the *cvff* force field of Dauber-Oguthorpe et al. [22]. This force field is the standard one used by the Discover 2.7 package, a component of Insight II of Biosym [23]. The same package was used for setting up the standard geometries for molecular graphics as well as the MD calculations. The dielectric constant was set to unity and the termination criterion for minimization was of 0.4 kJ mol^{-1} nm^{-1}. MD was performed for 100 or 200 ps at 300 K, with the first 6 ps of progressive heating from 0 to 300 K, by using the standard Discover procedure. Motivation for using the standard *cvff* force field comes from its very wide use in many different laboratories and its "general purpose" construction, which includes all necessary parameters. As for all other methods, its applicability and limits have to be verified case-by-case.

3. Results and discussion

Configuration of the acetal carbon atom.—The *R* and *S* configurations of the acetal carbon atom of pyruvate linked to O-4 and to O-6 of the methyl α-D-galactopyranosyl residue are shown as **1**-*R* and **1**-*S* in Fig. 1.

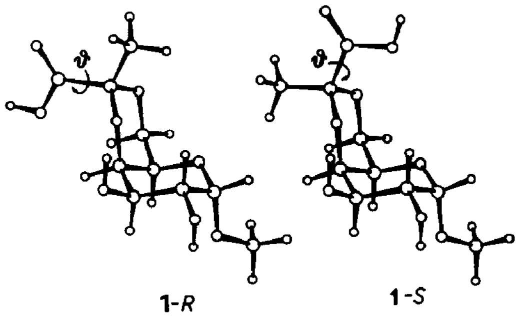

Fig. 1. A view of the conformational minima of methyl 4,6-*O*-(1-carboxyethylidene)-α-D-galactopyranoside (**1**): **1**-*R* has the *R* absolute configuration at the acetal carbon atom, and **1**-*S* has the *S* absolute configuration at the acetal carbon atom. The notation for the torsional angles is also shown.

The two structures were energy-minimized according to the methods described in the previous section. Each structure may be further characterized by two opposite orientations of the hydroxyl of the carboxylic group; one "internal" (*in*), i.e., towards the 1,3-dioxane (**2**) ring, the other "external" (*ex*), i.e., out of the 1,3-dioxane ring. Some details of the rotational barriers and the dynamics about the $>C(CH_3)-CO_2H$ bond (defined by the torsional angle θ) are given below. Here we will refer to the energy values of the two isomeric forms *R* and *S*, with the carboxylic group oriented in either one of the two minima, as "*in*" and "*ex*", respectively.

Most of the interactions contributing to the stabilization of the *R* and *S* configurations occur between the atoms of the methyl and the carboxyl groups and those of the 1,3-dioxane ring. Therefore, in order to understand the effect of the local environment on the acetal carbon, simpler basic compounds were also studied. The two basic compounds lacking in the sugar moiety are 2-methyl-2-carboxyl-1,3-dioxane (**3**) or 1,3-dioxane (**2**) (see Fig. 2). Here the carbon atom is no longer asymmetric, as the two forms, previously identified as *R* and *S* for **1**, are

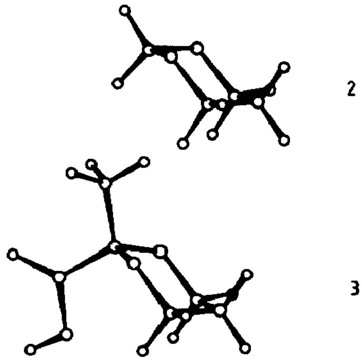

Fig. 2. Model molecular "portions" used for the evaluation of the conformational stability of the *R* and *S* forms: 1,3-dioxane (**2**) and 2-carboxyl-2-methyl-1,3-dioxane (**3**).

Table 1
Relative stabilities of individual structures of 3 calculated by different methods [a]

Method	R_{ex}-3	R_{in}-3	S_{ex}-3	S_{in}-3
AM1	0	13.3	15.8	15.8
MM (*cvff*)	0	6.52	7.32	9.83
STO-3G	0	6.9	9.58	8.75
3-21G	0	13.5	19.8	19.3

[a] All geometrical parameters were optimized. Energy values are in kJ mol^{-1}.

dynamically interconvertible to each other. For the purpose of comparison with the related *R* and *S* forms of **1**, in the following we conventionally maintain the structural distinction as *R* and *S* also for these compounds.

The total energy of each of the four conformers of **3** has been calculated using different methods (AM1, two different *ab initio* procedures and the more simplistic MM) by minimizing all geometrical parameters. The relative energies of each form with respect to the R_{ex} form are given in Table 1. Although some quantitative differences are evident in the results from the different methods employed, nonetheless in all cases the R_{ex} form was the most stable one.

More interestingly, despite the different levels of sophistication attributed to the four theoretical approaches, the same order of stability ($R > S$) was obtained. This conclusion is not only in agreement with some experimental observations [13,14], but can be fruitfully taken as a reason for using simpler methods such as MM *cvff*, at least for part of the present investigation. In fact, the AM1 method gives an energy difference between *R* and *S* close to that calculated with the *ab initio* approach using the 3-21G basis set, while the MM *cvff* and the STO-3G *ab initio* give comparable results.

In conclusion, the order of stability of individual forms (*ex* and *in*) was as follows: $R_{ex} > R_{in} > S_{in} > S_{ex}$. The different computational approaches produce some difference in the relative stability of S_{in} vs. S_{ex}, which ranges, however, within 2.5 kJ/mol. This difference does not modify the general conclusion that the *R* forms are always more stable than the *S* forms, a result in full agreement with the quoted NMR data [13,14].

The effect of carboxylate ionization.—The relative stability of the *R* vs. *S* form has also been evaluated with respect to the ionisation of the carboxyl group in **3** and in the presence of the galactopyranosidic ring. With **3**, no major changes in the order of stability were observed with the ionisation of the CO_2H group. As shown in Table 2, with all of the methods used the difference in stability between the *R* and *S* configuration in **3** becomes even more significant. It should also be noted that the *ex* and *in* forms become identical after ionisation (see Table 2).

An increase in stability for the R_{ex} configuration vs. other forms is also found for the cyclic acetal linked to a saccharide: the α-D-galactopyranoside unit has been considered in the calculations (Table 3). The S_{in} configuration becomes more stable than S_{ex} in both methods considered (AM1 and MM), and this is probably due to weak hydrogen-bonding between the OH group (of CO_2H) and O-5 of the

Table 2
Effect of ionization of the CO_2H group on the relative energies of 3 [a]

Method		R_{ex}-3	S_{ex}-3	S_{in}-3
AM1	ionised	0	23.9	
	neutral	0	15.8	15.8
MM (*cvff*)	ionised	0	31.5	
	neutral	0	9.58	8.70
STO-3G	ionised	0	36.2	
	neutral	0	7.32	9.83

[a] All geometrical parameters were optimised. Energy values are in kJ mol^{-1}.

galactose ring. In fact, in the S_{in} form, the acetal hydroxyl group directed toward that atom may be as close as 0.29 nm.

Orientation of methyl and carboxyl groups.—When analysing the possible reasons for the higher stability of the *R* configuration, a strong repulsion between the bulky carboxylic group and the ring might first have been expected. However, and in contrast, the results indicate not only that the repulsion between a methyl group and 1,3-dioxane ring is lower in the *R* than in the *S* form, but also that the dipole–dipole interaction of a CO_2H group with C–O bonds of the 1,3-dioxane ring are more favourable in the *R* form.

Some variations are shown by the values of the energetic barriers of the internal rotation in 3. Figs. 3 and 4 show the energy profiles along torsion angle θ for the rotation about the carboxyl group (with the zero value set for carbonyl oxygen eclipsed with the methyl group). In both *R* and *S* configurations, the barriers do not exceed 8.4 kJ/mol, with all other geometrical features being completely relaxed during the rotation about θ. However, further critical attention is warranted to evaluate the peculiar difference between the energy profiles obtained for the *R* and *S* forms as well as for the profiles calculated by the MM or AM1 methods.

According to MM calculations (Fig. 3), in the *R* configuration there are three minima located at $\theta \approx 0$, 113, and $-113°$, the former having an energy ca. 3 kJ/mol higher than the other two symmetric and equivalent minima. These

Table 3
Effect of the galactopyranosyl ring on the relative stability of structures [a] of 3

Method	R_{ex}-3	R_{in}-3	S_{ex}-3	S_{in}-3
AM1				
without G	0	13.3	15.8	15.8
with G	0	18.7	20.6	19.0
MM (*cvff*)				
without G	0	6.52	7.32	9.83
with G	0	7.74	7.49	4.69

[a] All geometrical parameters were optimised. Energy values are kJ mol^{-1}.

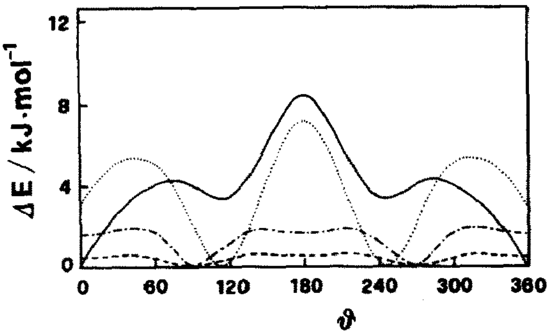

Fig. 3. Conformational-energy profile for the rotation of the carboxyl group of 3 about the torsional angle θ, with a fixed conformation of the 1,3-dioxane ring, calculated by the MM method. Curves refer to the uncharged carboxyl group for the S form (———) and for the R form (······) and to the charged carboxylate group for the S form (— — —) and for the R form (—·—·—).

minima are separated by barriers of $E_{(\theta \approx 60° \text{ and } -60°)} = 5$ kJ/mol and a larger one of $E_{(\theta = 180°)} = 7.1$ kJ/mol, corresponding to the *cis* orientation of the OH group with the methyl group. The torsional stability for the S configuration is reversed (Fig. 3); in this case the absolute minimum is at $\theta = 0°$ and the other two minima (at $\theta \approx 120$ and $-120°$) are ca. 3.3 kJ/mol higher. The variation in the barriers also appears to be a consequence of a superimposed potential, symmetrically centred at 180°, which unequivocally arises from the more-favourable interaction of the OH group with the 1,3-dioxane oxygen atoms in the S form when the torsional angle is at $\theta = 0°$. In all cases, the rotation of the CO_2H group is affected by the three-fold symmetry effectively generated by the acetal carbon atom.

The description of profiles given by the AM1 method is quite different (Fig. 4) for the R_{ex} form for which the absolute minimum lies at $\theta = 0°$, while the secondary minimum is at $\theta = 180°$. Therefore, a two-fold symmetry appears as a major difference from the MM calculations. These differences are mainly caused by electron delocalization (namely polarization), which is well described by AM1 but is not considered in MM.

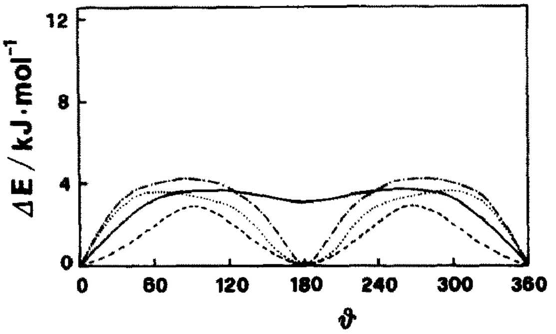

Fig. 4. Conformational-energy profile for the rotation of the carboxyl group of 3 about the torsional angle θ, with a fixed conformation of the 1,3-dioxane ring, calculated by the AM1 method. Symbols as in Fig. 3.

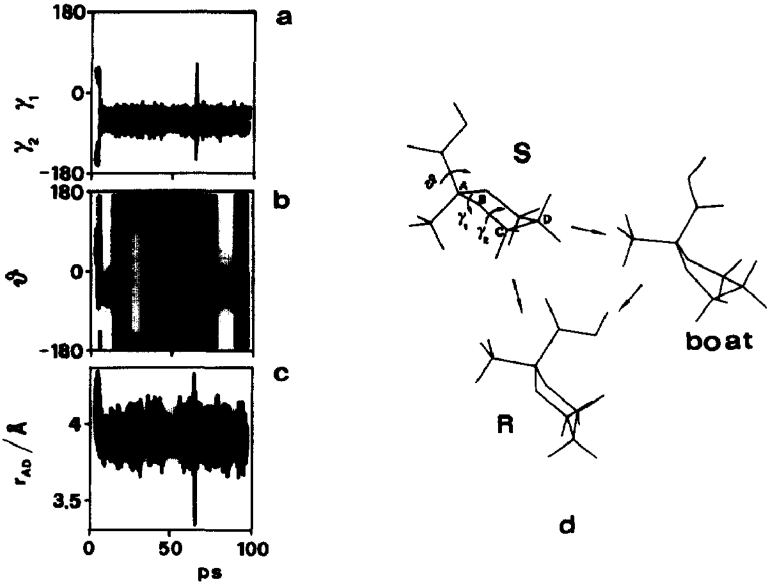

Fig. 5. Molecular dynamics simulation of the conformation of 3: (a) time dependence of the torsional angles γ_1 and γ_2; (b) time dependence of the torsional angle θ; (c) time dependence of the distance r_{AD}; (d) "snapshots" of the molecular structures obtained by molecular dynamics indicating the interconversion among the *S*, *R*, and boat forms.

Detailed studies on the energetics of the rotation of carboxylic group are given by Newton and Jeffrey [24], by using *ab initio* (4-31G) calculations. Their profiles on different molecules are very similar to those obtained here for the *R* form by using AM1.

As far as the ionised form is concerned, the differences found between the profiles obtained with the AM1 and MM methods become smaller. MM calculations suggest that a CO_2^- group may rotate almost freely along θ with very small barriers (0.8–1.0 kJ/mol) in both *R* and *S* forms, with a preferential orientation at $\theta = 90°$ (Fig. 3), that is with the CO_2^- plane ortogonal to the plane defined by the carbon atoms of the CH_3–C–CO_2^- group. According to AM1 calculations the heights of the barriers in the ionised form remain low and similar to those found for the neutral form, retaining the overall two-fold symmetric profile as a reasonable consequence of the ionisation of the carboxylic group (Fig. 4).

Molecular dynamics.—Simulations by molecular dynamics have been carried out starting from the *S* form of the molecule of 3, which, after a short time (ca. 6 ps), turns into the *R* form by flipping about the two torsional angles γ_1 and γ_2 (Fig. 5a). This is clearly seen from the time dependence, for example of γ_1, which shifts from the value of +60° (namely that characterizing the *S* form) to −60° (that is the value characterizing the *R* form. There is also evidence for some intermediate states arising from Fig. 5c, in which the parameter r_{AD} is plotted vs. t, with r_{AD} being not only osciliatory about the mean value, but also briefly reaching the value characteristic of the boat form during the *S* to *R* interconversion. In fact, the

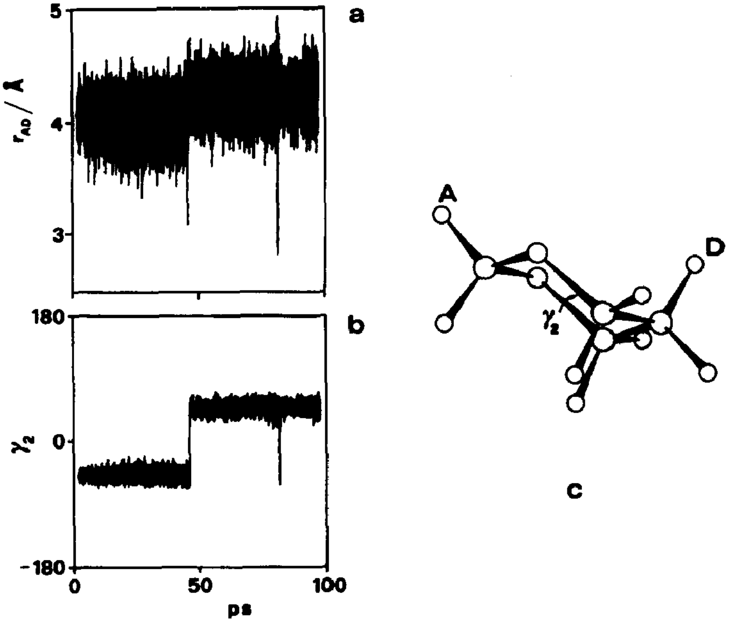

Fig. 6. Molecular dynamics simulation of the conformation of **2**: (a) time dependence of the distance r_{AD}; (b) time dependence of the torsional angle γ_2; (c) "snapshot" of the stable structure of **2**.

'snapshot' of the molecular structure taken at ca. 66 ps (which corresponds to the shortest r_{AD} value) gives the boat form of Fig. 5d. However, this structure exists only for a short time, the major residential time corresponding to the structure around the *R* configuration. Concerning the carboxylic group, there is almost complete conformational freedom for rotation of the CO_2H group about the torsional angle θ (Fig. 5b), although some favoured states are also shown. In fact, a modest predominance (for short periods of time) of torsional values in the range of $\theta = 0–90°$ and at ca. 180° is observed.

All these results are even more significant when compared with parallel results (Fig. 6) obtained on the simpler 1,3-dioxane ring (**2**), which spends roughly half of the time in the *R* form ($\gamma_2 = +60°$) and half in the *S* form ($\gamma_2 = -60°$), as would be expected on the basis of the symmetry of the molecule (the two conformations in this case are identical). The interconversion between *R* and *S* as well as the existence of some half-chair to quasi-boat forms is clearly seen from the large oscillation and the time dependence of the distance r_{AD}, that is the distance between the two hydrogen atoms on the same side of the plane of the ring (Fig. 6a). Interestingly, however, the frequency with which **2** flips during the limited time of observation appears to be comparable to that of **3**. The number of interchanges obviously depends on the energy barriers for flipping.

As regards the glycoside **1**, the presence of the α-D-galactopyranosyl ring greatly modifies the dynamic behaviour of the **3** moiety. The most important, although obvious, characteristic is the absence of the flipping of the 1,3-dioxane ring: that is the angles γ_1 and γ_2 oscillate continuously within a constant range of values (Fig.

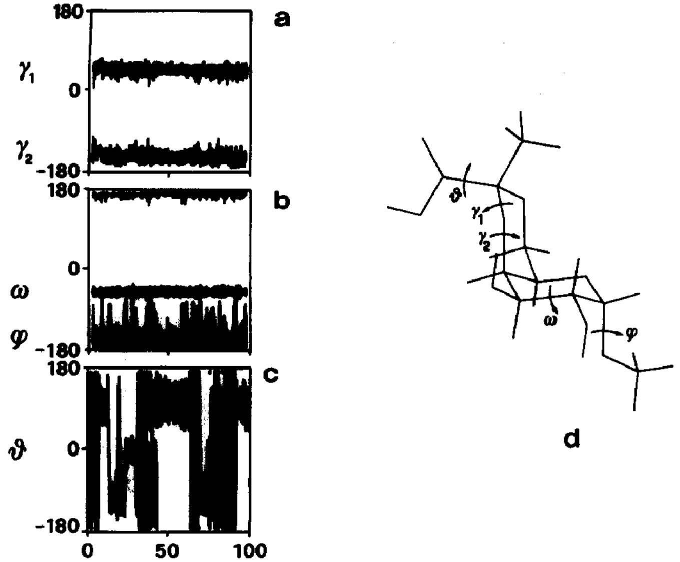

Fig. 7. Molecular dynamics simulation of the conformation of **1**: (a) time dependence of the torsional angle γ_1 and γ_2; (b) time dependence of the torsional angles ω and ϕ; (c) time dependence of the torsional angle θ; (d) "snapshot" of the molecular structure of **1**.

7a), which is even more restricted than that observed for **3**. This is due to the restriction of configuration at C-4 of the α-D-galactopyranosyl ring; that is, the inversion of the ring of **3** can could occur only by reversing the configuration at C-4, a consideration that applies to both the *R* and the *S* forms of **1**.

At the same time, the dihedral angle ω, which has been simply taken to follow the changes of the pyranoid-ring geometry (Fig. 7b), also oscillates within a very restricted range (at ca. −60°). While the absence of interconversions from the ${}^1C_4$ form to the ${}^4C_1$ form appears a consequence, again, of the attached moiety of **3** attached to the galactopyranosyl ring, the decrease of fluctuations seems somehow excessive at first. Indeed, a possible source of such increased rigidity against interconversion may arise from the *cvff* force-field itself (especially for the out-of-plane vibrations). This hypothesis is supported in part by the low frequency of the $R \Leftrightarrow S$ interconversions of **2** already discussed above.

Two other considerations emerge from analysis of the dynamic freedom of torsional angles in **1**. The first is that the rotation of the carboxylic group (Fig. 7c), although it still turns through 360°, appears more hindered, that is, the rotational barriers are higher than those in the related structure **3**. Here, three favoured states of the θ intervals may be seen apart from the overall (but restricted) rotation in a certain period of the time explored, namely at ca. 0, 120, and −120°. This corresponds roughly to the profile shown in Fig. 3 for the *R* form of **3**. It is not conceivable that this hindrance is caused by the effect of repulsion potentials from the glycosidic ring; more likely, the larger amplitude of fluctuation of γ_1 and γ_2 in **3** favours concerted oscillations also in the CO_2H group.

The second consideration concerns the rotational angle ϕ (defined with respect to C-2–C-1–O–C-4′ in Fig. 7b). This angle formally corresponds to one of the two conformational angles (ϕ and φ) that allow polysaccharides to assume different chain conformations. Its variance shows a wide minimum centered at ca. $-140°$ (corresponding to ca. $-20°$ in the other H-1–C-1–O–C-4′ notation used), as is usually found from conventional molecular mechanics on α-galactosides.

4. Conclusions

The higher stability of the *R* form for the acetal carbon of a pyruvate-linked to O-4 and O-6 of a galactose has been confirmed by all of the theoretical methods used and for all of the simple model molecules investigated. This configuration corresponds to the structure having the methyl and carboxyl groups in the equatorial and in the axial dispositions, respectively. The solvent effect, as modelled in a preliminary way in this study, slightly decreases the stabilization of the *R* form vs. the *S* form; however, the *R* form still remains the most stable one.

Methods of different degrees of sophistication have been compared in order to assess the usefulness of expensive computer time. Two *ab initio* methods, the AM1 and the MM*cvff* methods give the same conclusions, although differing in numerical values. The "quality" of the *cvff* force field seems to need further testing, especially as far the interconversion processes and stereoelectronic effects are concerned. MD has shown a decrease of flexibility occurring in both the flipping of the skeletal and the carboxyl group rotation, with increasing molecular size (by attaching the galactopyranosyl residue). More concerns arise from study of the favoured conformation of the carboxylic group. Not only do the two methods explored give different stabilities, but it appears clear that torsional potentials have different symmetry about the rotational angle by the two methods. This apparent contradiction may be partially reconciled by considering that the energy profiles of the ionised form are quite smooth in all cases, although they become more pronounced for the unionised form.

The previous finding [11] of a change in conformation of the galactoglucan, induced by removal of pyruvate substituents from the native polysaccharide, may now be reconsidered on stronger grounds, by introducing a proper role of the pyruvate group in calculations of the chain conformation. Further studies are now in progress on this line and on the MD modelling of the solvent (both with discrete and the continuum approaches), which may provide additional evidence for possible preferential conformations of the carboxylic group under different states of ionisaton.

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References

[1] M. McNeil, J. Darvill, A.G. Darvill, P. Albersheim, R. van Veen, P. Hooykaas, R. Schilperroort, and A. Dell, *Carbohydr. Res.*, 146 (1986) 307–326.

[2] J. Glazebrook, J.W. Reed, T.L. Reuber, and G.C. Walker, *Int. J. Biol. Macromol.*, 12 (1990) 67–70.

[3] H.C.J. Canter-Cremers, M. Batley, J.W. Redmond, A.H.M. Wijfjes, B.J.J. Lugtenberg, and C.A. Wijffelman, *J. Biol. Chem.*, 266 (1991) 9556–9564.

[4] J.A. Leigh and D.L. Coplin, *Annu. Rev. Microbiol.*, 46 (1992) 307–346.

[5] J.A. Leigh, J.W. Reed, J.F. Hanks, A.M. Hirsch, and G.C. Walker, *Cell*, 51 (1987) 579–587.

[6] I.W. Sutherland, *Adv. Microbial Physiol.*, 23 (1982) 80–142.

[7] M.S. Kuo and A.J. Mort, *Carbohydr. Res.*, 145 (1986) 247–265.

[8] R.I. Hollingsworth, M. Abe, F.B. Dazzo, and K. Hallenga, *Carbohydr. Res.*, 134 (1984) c7–c11.

[9] M. Kuo, A.J. Mort, and A. Dell, *Carbohydr. Res.*, 156 (1986) 173–187.

[10] T. Coviello, V. Crescenzi, M. Dentini, and A. Cesàro, *Polymer*, 31 (1990) 834–839.

[11] A. Cesàro, G. Tomasi, A. Gamini, S. Vidotto, and L. Navarini, *Carbohydr. Res.*, 231 (1992) 117–135.

[12] K.P. Shatwell, I.W. Sutherland, I.C.M. Dea, and S.B. Ross-Murphy, *Carbohydr. Res.*, 206 (1990) 87–103.

[13] P.J. Garegg, P.E. Jansson, B. Lindberg, F. Lindh, J. Lonngren, I. Kvarnstrom, and W. Nimmich, *Carbohydr. Res.*, 78 (1980) 127–132.

[14] P.A.J. Gorin, M. Mazurek, H.S. Duarte, M. Iacomini, and J.H. Duarte, *Carbohydr. Res.*, 100 (1982) 1–15.

[15] M.D. Newton, W.A. Latham, W.J. Hehre, and J.A. Pople, *J. Chem. Phys.*, 52 (1970) 4064–4072.

[16] J.S. Binkley, J.A. Pople, and W.J. Hehre, *J. Am. Chem. Soc.*, 102 (1980) 939–947.

[17] K.B. Wiberg and M.A. Murcko, *J. Am. Chem. Soc.*, 111 (1989) 4821–4828.

[18] M. Peterson and R. Poirer, MONSTERGAUSS 87 program, Toronto, 1987.

[19] M.J.S. Dewar, E.G. Zoebisch, E.F. Healy, and J.J.P. Stewart, *J. Am. Chem. Soc.*, 107 (1985) 3902–3909.

[20] J.J.P. Stewart, *J. Comp. Mol. Design*, 4 (1990) 1–105.

[21] I. Tvaroska and J.P. Carver, *J. Chem. Res.*, (*S*), (1991) 6–7.

[22] P. Dauber-Osguthorpe, V.A. Roberts, D.J. Osguthorpe, J. Wolff, M. Genest, and A.T. Hagler, Proteins: Structure, Function Genetics, 4 (1988) 31–47.

[23] Insight II. User Guide, BIOSYM Technologies (1990).