Density Functional Study of the Conformational Space of $^4C_1$ D-Glucuronic Acid

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The conformational space of $^4C_1$ α- and β-D-glucuronic acid was scanned by HF/3-21G(p) calculations followed by optimization of the 15 most stable structures for each, using the B3LYP density functional theory method in conjunction with a diffuse polarized valence triple-ζ basis set. We found a general preference of the α anomers in the isolated molecules in agreement with the large endo-anomeric hyperconjugation effects in these structures. From the other intramolecular interactions (exo-anomeric hyperconjugation, hydrogen-bonding, dipole−dipole, and steric interactions), the effect of the hydrogen bonding is the most pronounced and plays a major role in determining the stability order within the α and β series. The most stable conformer of both α and β $^4C_1$ D-glucuronic acid is the structure with the maximum number (5) of intramolecular hydrogen bonds. Introduction of solvent (water) effects by the SCI-PCM model resulted in two characteristic changes of the energetic properties: the gas-phase stability order changed considerably, and the energy range of the 15 most stable conformers decreased from 30 to 15 kJ/mol. The geometrical parameters reflect well the superimposed effects of hyperconjugation and hydrogen-bonding interactions. Most characteristics are the variations of the C−O bond distances (within a range of 0.04 Å) upon the combined intramolecular effects.

Introduction

The biological functions of carbohydrates are strongly related to their conformational properties.[1,2] These properties include the structure of the pyranose rings ($^4C_1$ chair, $^1C_4$ chair, skew, or skew-boat forms) as well as the orientation of the hydroxy groups and other substituents. The OH rotation regulates the intra- and intermolecular hydrogen-bonding interactions, which play a major role in the relative stability of a particular conformer and in the interactions of carbohydrates with other molecules.

Due to the many geometrical variables, the conformational space of carbohydrates is very rich. On the basis of the three-fold rotational freedom of the OH and CH_2OH side-chains, in principle, $3^5 = 729$ different rotamers can be generated for a given pyranose ring of glucose and related monosaccharides. Many of these rotamers, however, are not stationary points on the potential energy surface of the molecule. A recent molecular mechanics search of the $^1C_4$ α-L-fucose by the systematic unbounded multiple minimum (SUMM) technique[3] resulted in only 17 minima on the potential energy surface.[4] Although the deficiencies of the force field method may omit several real minima, the latter study indicates that the number of stable carbohydrate structures is considerably less than assumed from the rotational freedom of the OH and CH_2OH groups. Responsible for this are the strong attractive forces between the hydroxy hydrogens and oxygens, which destabilize OH orientations other than those leading to hydrogen bonding.

We have been interested in the unique structural properties of hyaluronan, that ubiquitous high-molecular-weight polysaccharide, which plays an important role, among others, in eye-tissues,[5] synovial fluid, cartilage, and pericellular spaces of the human body.[6] While the structural properties of hyaluronan have been thoroughly investigated,[6−11] relatively little is known about its building-blocks, the D-glucuronic acid and *N*-acetyl-D-glucosamine monosaccharides. Of the two, only the crystal structure of *N*-acetyl-D-glucosamine has been determined by X-ray diffraction,[12] whereas a single theoretical study of hyaluronan fragments (focusing on the di- and trisaccharides) reported a few structural and electrostatic properties of the monosaccharides.[13] That latter study dealt with only one conformer of both monoanionic β-D-glucuronic acid and neutral *N*-acetyl-β-D-glucosamine using low-level AM1 and HF/3-21G computations. Hence, the conformational space and the nature of intramolecular interactions in these monosaccharides are still unknown.

The present paper contributes to the chemistry of D-glucuronic acid. Our goal is to elucidate the conformational space of the $^4C_1$ α and β anomers using advanced quantum chemical methods. Characteristics of the $^4C_1$ chair are the carboxyl and, except for the anomeric O_1H group in the α anomers, the hydroxy groups occupying equatorial positions, whereas in the $^1C_4$ chair they are switched to the sterically less favored axial positions. Note that D-glucuronic acid is present in the $^4C_1$ chair form in hyaluronan.[7−9]

To locate all of the possible minima, we scanned the conformational space of the title compound systematically by HF/3-21G(p) calculations. The geometries of the minima were further optimized using density functional theory (DFT). The change of the energy properties of the conformers from the isolated molecule to aqueous solution was investigated by the SCI-PCM solvation model.[14] In addition, we analyzed in detail the intramolecular interactions in selected structures of the lowest energy.

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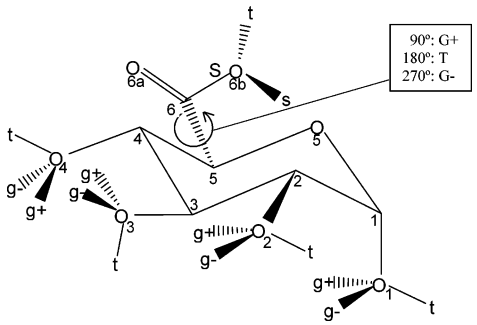

Figure 1. Designation of the OH and COOH rotation is based on the $C_{n+1}-C_n-O-H$ and $C_5-C_6-O_{6b}-H$ idealized torsion angles, respectively: t (anti, 180°); s (syn, 0°); g+ (gauche clockwise, 60°); g− (gauche counterclockwise, −60°). The orientation of the COO group is defined on the basis of the $O_5-C_5-C_6-O_{6b}$ angle: S (syn, 0°); G+ (90°); T (anti, 180°); G− (270°).

Computational Details

The initial geometries of the α and β-D-glucuronic acid rotamers were generated by a homemade script written in freepascal under Linux. Considering three-, four-, and two-fold rotations of the OH, COO, and carboxylic OH groups, respectively, this means altogether 648 structures for each anomer. The pre-optimizations performed at the HF/3-21G(p) level led to 58 and 62 different minima in the space of the α and β anomers, respectively. The computed HF/3-21G(p) vibrational frequencies of these structures were all positive. The first 15 lowest-energy minima of each anomer were further optimized at the B3LYP/6-311G** level. To better account for hydrogen bonding, the basis sets of the oxygens and hydroxy hydrogens were extended by diffuse functions. The importance of diffuse functions for such studies is in providing space for the electrons far from the nuclei and thus improving the performance of the long-range part of the correlation and exchange functionals for the OH···O interactions.[15,16] From the two sets, the diffuse functions on oxygen are essential for carbohydrate energetics, whereas for those on hydrogen a marginal effect has been reported.[17] Our basis set including diffuse functions is denoted in the following as 6-311(++)G**.

The solvent effects of water were taken into account by the SCI-PCM model[14] at the B3LYP/6-311(++)G** level. Because our geometry optimizations within the SCI-PCM scheme failed for many conformers, the solvation energies were computed for the B3LYP/6-311(++)G** optimized geometries. All of the calculations were performed using the Gaussian 98 program.[18]

Results and Discussion

The conformers are characterized by the following angles of torsion, $C_{n+1}-C_n-O-H$, $O_5-C_5-C_6-O_{6b}$, and $C_5-C_6-O_{6b}-H$, as defined in Figure 1. These angles of torsion were labeled in agreement with general conventions.[4,16] The relative energies of the 15 lowest-energy conformers of α- and β-D-glucuronic acid are given in Table 1.

Before a detailed analysis of the most characteristic conformers, we want to comment briefly on the performance of the HF/3-21G(p) level in the conformational analysis. These calculations predicted correctly the **A1** conformer being the global minimum and the overall larger stability of the α anomers (cf., Table 1). The relative energies of the conformers have magnitudes similar to the B3LYP values. However, the calculations at the HF level systematically underestimate the stabilities of higher-energy conformers with respect to the global minimum. They also interchange the stability order of several close-energy conformers as compared to the B3LYP results. The most conspicuous error appears in the overstabilization of the **A11** conformer. This error can be related to the (T)(t) orientation of the COOH group as witnessed, although to a smaller extent, by the **A14** and **A15** conformers as well. In the (T)(t) COOH orientation, a considerable repulsion between the O_5 and O_{6a} lone pairs can be expected. This repulsion is probably underestimated in the HF model as a consequence of the contracted valence electron density due to the neglect of electron correlation. The contracted valence electron density is also responsible for the shorter hydrogen bonds in the HF/3-21G(p) structures.

TABLE 1: Relative Energies of Selected Conformers of D-Glucuronic Acid

		ΔE^b		
no.	characterization[a]	HF	B3LYP	SCI-PCM
	α-D-Glucuronic Acid			
A1	(g−)(g−)(g+)(g−)(S)(s)	0.0	0.0	3.7
A2	(t)(g−)(g+)(g−)(S)(s)	14.1	7.6	1.5
A3	(g−)(g−)(g+)(g−)(S)(t)	14.5	8.5	9.8
A4	(t)(g+)(g+)(g−)(S)(s)	17.8	11.6	0.0
A5	(t)(g−)(g+)(g−)(S)(t)	25.2	13.9	8.2
A6	(t)(g+)(t)(t)(G+)(t)	26.5	15.2	7.5
A7	(t)(g+)(t)(g−)(S)(s)	22.6	15.6	1.0
A8	(t)(g+)(t)(t)(G−)(t)	26.1	16.1	8.4
A9	(t)(g+)(g+)(g−)(S)(t)	26.1	16.3	6.8
A10	(t)(g+)(t)(t)(T)(s)	20.9	16.4	3.4
A11	(g−)(g−)(g+)(g−)(T)(t)	17.0	16.9	12.4
A12	(t)(g+)(t)(g−)(S)(t)	30.3	19.0	8.2
A13	(g−)(t)(t)(g−)(S)(s)	31.1	21.4	8.1
A14	(t)(g−)(g+)(g−)(T)(t)	27.8	21.7	10.3
A15	(t)(g+)(g+)(g−)(T)(t)	29.4	24.0	8.8
	β-D-Glucuronic Acid			
B1	(g+)(g−)(g+)(g−)(S)(s)	27.4	9.2	9.1
B2	(g−)(g−)(g+)(g−)(S)(s)	31.4	9.9	4.3
B3	(t)(g−)(g+)(g−)(S)(s)	32.7	11.6	2.7
B4	(t)(t)(g+)(g−)(S)(s)	37.4	15.8	2.9
B5	(t)(t)(t)(g−)(S)(s)	38.6	17.6	3.1
B6	(t)(g−)(g+)(g−)(G+)(t)	44.9	18.5	9.9
B7	(t)(t)(t)(t)(G+)(t)	45.2	18.6	10.3
B8	(g−)(t)(g+)(g−)(S)(s)	41.4	18.9	6.6
B9	(g−)(g−)(g+)(g−)(S)(t)	47.0	19.5	10.6
B10	(t)(t)(t)(t)(G−)(t)	45.2	19.5	10.9
B11	(t)(t)(t)(t)(T)(s)	39.9	19.6	5.9
B12	(g+)(g−)(g+)(g−)(S)(t)	46.3	20.1	15.1
B13	(t)(t)(g+)(g−)(S)(t)	47.1	20.7	9.5
B14	(g−)(t)(t)(g−)(S)(s)	42.9	21.2	7.1
B15	(t)(g−)(g+)(g−)(T)(t)	47.4	26.8	12.0

[a] The designators used for characterization of the conformers are defined in Figure 1. The order of the code letters follows the clockwise sequential numbers of the oxygen atoms. [b] Relative energies are given in kJ/mol. In the HF computations the 3-21G(p) basis, in the B3LYP computations the 6-311(++)G** basis set was used. The SCIP-CM energies were obtained in single-point calculations on the B3LYP/6-311(++)G** optimized geometries using the same theoretical level. The B3LYP and SCI-PCM data include zero-point vibrational energy corrections obtained from B3LYP/6-311(++)G** frequency calculations.

The relative stability of carbohydrate conformers is determined by several weak interactions, viz., hydrogen-bonding, dipole−dipole, and hyperconjugation interactions as well as steric effects. The hydrogen-bonding and the most important dipole−dipole interactions have the strongly polar OH groups in common. Strong attractive dipole−dipole interactions can be expected, for example, in the hydrogen-bonding ···O−H···O−H··· chains.

The most important hyperconjugation effects of carbohydrates appear in the $O_5-C_1-O_1$ moiety. The charge transfer from the lone pairs of O_5 to the antibonding C_1-O_1 orbital ($n_{O_5} \rightarrow \sigma^*_{C_1-O_1}$, endo-anomeric effect) takes place when the O_1H group

TABLE 2: Second-Order Perturbation Energies (ΔE) Characterizing the Endo- and Exo-anomeric Effects[a]

conformer	ΔE_{endo}	ΔE_{exo}
A1	45.2	24.9
A2	43.1	70.5
A3	48.5	23.7
A4	49.4	63.8
A5	46.7	64.9
A10	58.3	56.1
B1	13.2	27.0
B2	12.8	73.8
B3	14.2	71.4
B4	13.5	65.8
B5	13.5	65.0
B11	16.2	51.2

[a] The second-order perturbation energies (kJ/mol) of the $n_{O_5} \rightarrow \sigma^*_{C_1-O_1}$ and the $n_{O_1} \rightarrow \sigma^*_{C_1-O_5}$ hyperconjugation interactions were obtained by NBO analysis.

occupies the axial position, whereas it is considerably reduced in the case of an equatorial O_1H position. The endo-anomeric effect is held responsible for the general preference of the α over β anomers of glucopyranoses in the gas phase.[19−21] This was also found in the present study for D-glucuronic acid, the most stable α conformer lying lower in energy by 9 kJ/mol than the most stable β one (cf., Table 1). The input of hyperconjugation effects can be estimated by a second-order perturbation analysis within the natural bond orbital (NBO) partitioning scheme.[22] The calculated second-order perturbation energies for the $n_{O_5} \rightarrow \sigma^*_{C_1-O_1}$ interaction in selected D-glucuronic acid conformers are given in Table 2. Although the hyperconjugation energies might be somewhat exaggerated (vide infra), they indicate a considerable energy gain of the α anomers from the endo-anomeric effect. This can overcompensate the unfavored steric interactions of the axial O_1H group.

Another important hyperconjugation interaction is the charge transfer from the lone pairs of O_1 to the antibonding C_1-O_5 orbital ($n_{O_1} \rightarrow \sigma^*_{C_1-O_5}$, exo-anomeric effect). The strength of this interaction depends on the orientation of the O_1H group: most favorable are the (t) and (g+) orientations in the α anomer, and the (t) and (g−) orientations in the β anomer. The data in Table 2 refer to some of these situations.

The computed structures of selected α and β anomers are depicted in Figures 2 and 3, respectively. The most stable $^4C_1$ structure is the α anomer with the maximum number (5) of intramolecular hydrogen-bonding interactions (**A1**, cf., Figure 2). In this structure, all of the hydroxy hydrogens are rotated clockwise and form hydrogen bonds with the neighboring oxygens. The preference of that clockwise hydrogen-bonding chain is manifested also in the most stable β anomer (**B1**, cf., Figure 3). In the chain, the OH groups on C_2, C_3, and C_4 as well as the COOH group serve simultaneously as donor and acceptor; hence the σ-cooperativity[23] can prevail in these structures.

Inspection of the hydrogen bonds in **A1** and **B1** reveals the strongest interactions exercised by the COOH moiety. The shortest hydrogen bond in these (and most other) conformers is formed between the carboxyl OH and pyranose oxygen O_5. We were curious about the relationship of this short 1.95-Å $H\cdots O_5$ distance, determined partly by the geometrical constraints of the ortho-positioned COOH group, to the optimal hydrogen bond in a relaxed structure. Alas, our literature search resulted in no hits for intermolecular hydrogen-bonding interactions between ether O and carboxyl H in the gas phase. Thus, we performed B3LYP/6-311(++)G** calculations on the model derived from A1 (the COOH group was released from C_5 permitting intermolecular interaction, and the free valences of the two fragments were filled by hydrogen). In this relaxed structure, we obtained an $H\cdots O$ distance of 1.79 Å, indicative of the optimal hydrogen-bond length between the pyranose O and carboxyl H. Accordingly, the geometrical constraints in D-glucuronic acid lead to a weaker $O_{6b}-H\cdots O_5$ interaction.

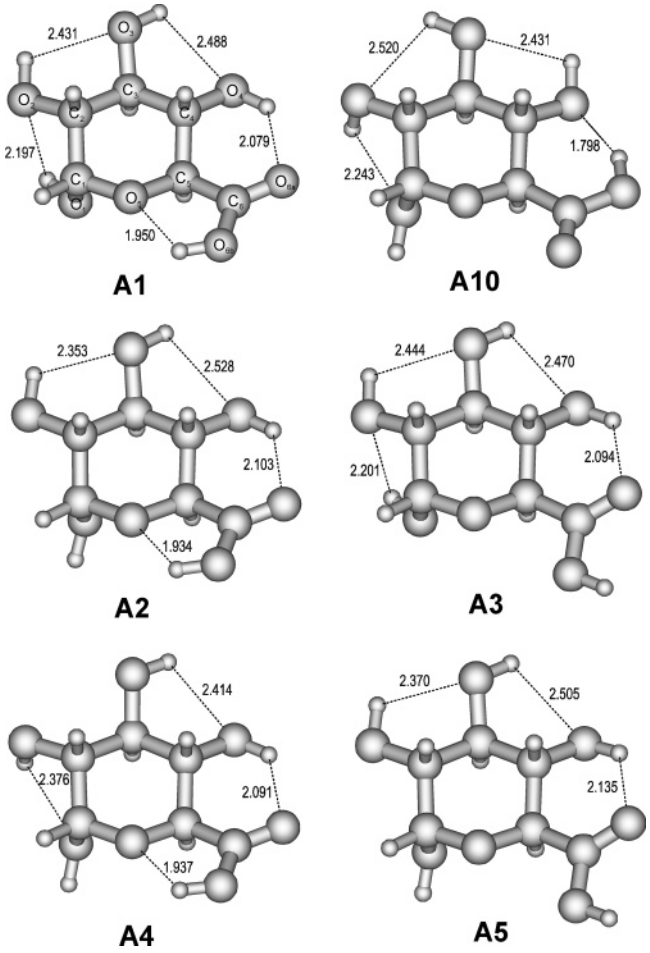

Figure 2. Selected conformers of α-D-glucuronic acid.

The COOH group participates in a second hydrogen-bonding interaction in which O_{6a} is the acceptor. These 2.1-Å $O_4H\cdots O_{6a}$ distances are shorter than the other intramolecular hydrogen bonds between the carbohydrate OH groups (cf., Figures 2 and 3) and imply a medium strength interaction. Note that the hydrogen bonds in **A1** and **B1** have similar magnitudes except $O_1H\cdots O_2$, which is considerably shorter in **A1** (2.20 Å) than in **B1** (2.36 Å). Besides the endo-anomeric effect, this stronger hydrogen bond may also contribute to the larger stability of the α anomer.

Next, we discuss another characteristic conformer pair, the structures with all of the hydrogen bonds directed counterclockwise (**A10** and **B11**, cf., Figures 2 and 3). They are higher in energy by 16 (**A10**) and 10 kJ/mol (**B11**) than the most stable **A1** and **B1** conformers, respectively. In contrast to the five hydrogen bonds in **A1** and **B1**, **A10** and **B11** contain only four such interactions. Yet, this alone cannot explain the preference of **A1** and **B1** because in the **A10** and **B11** structures the (t) orientation of O_1H permits a strong exo-anomeric hyperconjugation (cf., Table 2). Furthermore, the hydrogen bonds formed between $O_{6b}H$ and O_4 are very strong in **A10** and **B11**: these $H\cdots O_4$ distances of 1.8 Å are the shortest in the present carbohydrate structures. However, inspection of **A10** and **B11** in more detail reveals a strong steric repulsion between the lone

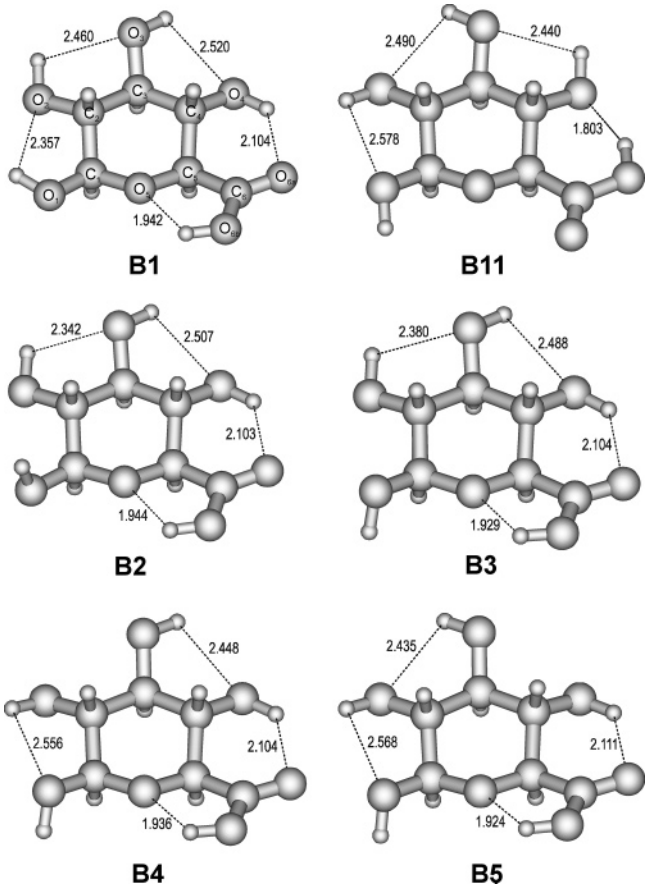

Figure 3. Selected conformers of β-D-glucuronic acid.

pairs of O_{6a} and O_5 as a consequence of the nearly parallel C_6-O_{6a} and C_5-O_5 bonds. This arrangement is probably determined by the strong $O_{6b}-H\cdots O_4$ hydrogen bonding at the other side of the COOH group. On the other hand, this steric interaction cancels the stabilizing effect of the $O_{6b}-H\cdots O_4$ hydrogen bond as indicated by the close relative energies of the **A6**, **A8** and **B7**, **B10** conformers to **A10** and **B11**, respectively. In the former structures, the COOH group is turned by 90° with respect to its orientation in **A10** and **B11**, thus avoiding both the $O_{6b}-H\cdots O_4$ hydrogen bonding and the $O_{6a}\cdots O_5$ repulsion.

A comparison of structures **A1**−**A5** reveals several characteristic intramolecular interactions, which help to understand the stability relations of the conformers. We inspect first **A2** and **A1** with **A2** being by 8 kJ/mol less stable than **A1**. The only difference between the two structures is the 120° rotation of the O_1H group, which has the following consequences: (i) the break of the $O_1-H\cdots O_2$ hydrogen bond, (ii) the disappearance of the repulsion between the lone pairs of O_1 and O_5, (iii) the emergence of large exo-anomeric effect, and (iv) considerable repulsion between the lone pairs of O_1 and O_2 in **A2**. Note that the repulsion between O_1 and O_5 in **A1** may be much smaller than that between O_1 and O_2 because of the larger distance and more relaxed relative orientation of O_1 and O_5. The only additional interaction with stabilizing energy gain in **A2** comes from the exo-anomeric effect: 45 kJ/mol, indicated by the second-order perturbation analysis (cf., Table 2). This value, however, might be somewhat exaggerated as (by adding the 8 kJ/mol preference of **A1**, cf., Table 1) an energy loss of 53 kJ/mol could unlikely come from the break of the $O_1-H\cdots O_2$ hydrogen bond and from the $O_1\cdots O_2$ repulsion.

The relative stability of **A3** with respect to **A1** is lower by 9 kJ/mol. A destabilization of **A3** comes from breaking of the $O_5\cdots H-O_{6b}$ hydrogen bond and from the added repulsion between the lone pairs of O_5 and O_{6b}. A considerable energy gain, compensating for most of the destabilization in **A3**, stems from the more favored syn orientation of the carboxyl OH with respect to $C=O_{6a}$. Our test calculations on formic acid revealed a preference of the syn conformer by 19 kJ/mol.

Somewhat different interactions are characteristic on the five most stable β anomers **B1**−**B5**. **B1**, **B2**, and **B3** are very close in energy and differ only in the relative orientation of O_1H. The weak (2.36 Å) $O_1-H\cdots O_2$ hydrogen bond in **B1** is broken in **B2** and **B3**, whereas in the latter structures an increased exo-anomeric effect appears. The (g−) orientation of O_1H (in **B2**) is slightly more favored than the (t) one (in **B3**), cf., Table 1. The counterclockwise rotation of the O_2H and O_3H groups in **B4** and **B5**, respectively, decrease the stability by about 5 kJ/mol with respect to **B3**. This might primarily stem from the replacement of the $O_2-H\cdots O_3$ hydrogen bond (2.38 Å) by the weaker $O_2-H\cdots O_1$ one (2.56 Å) in **B4** and **B5**.

Some information on solvent effects on the energy properties of D-glucuronic acid conformers can be extracted from our SCI-PCM results in Table 1. The data have certain limitations as, like any continuum solvation model, SCI-PCM cannot account for individual solute−solvent (e.g., hydrogen bonding) interactions: it describes only the general effect of a polar medium on the solute molecule.[24] Presently, the most suitable way to treat the complex hydration shell of carbohydrates[25] would be the application of the Car−Parrinello molecular dynamics method, which has been successfully applied in such studies.[26,27] Furthermore, we were not able to perform geometry optimizations within the SCI-PCM model; hence the geometry changes (and their energy consequences) in the polarizable solvent medium had to be neglected.

Nevertheless, our data reflect important trends upon the van der Waals interactions between the D-glucuronic acid conformers and the water medium. The most obvious effect in the data of Table 1 is seen in the general decrease of the relative energies in the series of the 15 conformers. The energy range of 30 kJ/mol in the gas phase decreased to 15 kJ/mol in the solution. Also, the energy order is changed considerably as van der Waals interactions between solute and solvent prefer structures with large polarizabilities. At the level of approximation used by us, the most stable conformer in aqueous solution is **A4**. Note that the general preference of the α anomers remained, although the energy differences with respect to the β ones decreased in agreement with previous reports on the decrease of anomeric effect by a polar solvent.[28−30]

The C−O and O−H bond distances of selected α and β conformers are collected in Table 3. These geometrical parameters are most sensitive to the above-discussed hydrogen-bonding and hyperconjugation interactions. Our data reflect the generally found lengthening of the donor O−H and acceptor C−O bonds upon hydrogen bonding.[31] The O−H lengthening originates from hyperconjugation effects associated with the hydrogen-bonding interaction: that is, the charge transfer from the lone pairs of the oxygen acceptor to the σ^*_{O-H} orbital, which weakens the proton donor O−H bonds in hydrogen-bonded systems.[22]

The magnitude of the geometrical effects correlates well with the O···H distances. Thus, O_1-H and O_4-H, forming stronger hydrogen bonds, are the longest (by a few thousandths of an angstrom) among the alcohol hydroxy groups. The carboxyl $O_{6b}-H$ lengthens by ca. 0.004 Å upon hydrogen bonding, as shown by comparing the conformers with (s) and (t) $O_{6b}H$ orientations. The O_1-H bonds change up to 0.005 Å. This

TABLE 3: Selected Geometrical Parameters[a] Characteristic of the Intramolecular Hydrogen-Bonding and Hyperconjugation Interactions

conformer	O_1-H	O_2-H	O_3-H	O_4-H	$O_{6b}-H$	C_1-O_5	C_1-O_1	C_2-O_2	C_3-O_3	C_4-O_4	C_6-O_{6a}	C_6-O_{6b}	C_1-H
A1	0.968	0.965	0.965	0.970	0.974	1.414	1.406	1.423	1.423	1.415	1.207	1.334	1.094
A2	0.963	0.965	0.964	0.969	0.974	1.438	1.396	1.412	1.424	1.416	1.207	1.336	1.092
A3	0.967	0.965	0.965	0.968	0.970	1.408	1.410	1.425	1.424	1.419	1.213	1.335	1.094
A4	0.963	0.965	0.965	0.969	0.973	1.426	1.409	1.411	1.415	1.416	1.206	1.336	1.092
A5	0.963	0.965	0.964	0.967	0.970	1.429	1.401	1.413	1.425	1.420	1.212	1.338	1.092
A10	0.964	0.967	0.965	0.965	0.978	1.409	1.418	1.420	1.423	1.432	1.196	1.346	1.092
B1	0.966	0.965	0.965	0.970	0.975	1.423	1.391	1.421	1.421	1.415	1.207	1.333	1.103
B2	0.965	0.966	0.965	0.970	0.975	1.448	1.378	1.413	1.422	1.415	1.207	1.334	1.095
B3	0.963	0.965	0.965	0.970	0.974	1.447	1.381	1.415	1.422	1.416	1.207	1.334	1.100
B4	0.963	0.963	0.965	0.970	0.974	1.437	1.390	1.416	1.415	1.416	1.206	1.335	1.100
B5	0.963	0.964	0.964	0.969	0.973	1.433	1.390	1.422	1.415	1.410	1.205	1.336	1.100
B11	0.965	0.964	0.965	0.966	0.978	1.423	1.391	1.421	1.422	1.431	1.196	1.346	1.103

[a] Bond distances are given in angstroms, and bond angles are in degrees.

magnitude, however, cannot be attributed solely to hydrogen bonding as the hyperconjugation of the C_1O_1H group with the ring C_1-O_5 bond also effects the O_1-H distance.[20]

The geometrical changes are more appreciable in the C−O bond distances. They lengthen up to 0.014 Å in the proton acceptor CO groups upon hydrogen-bond formation (cf., Table 3). The $C_6{=}O_{6a}$ double bond lengthens up to 0.011 Å in hydrogen bonding.

Differences in the C_1-O_1 and C_1-O_5 bond distances between the conformers can be interpreted as a consequence of composite hyperconjugation and hydrogen-bonding effects. In more detail, these are the $O_1-H\cdots O_2$, $O_2-H\cdots O_1$, and $O_{6b}-H\cdots O_5$ hydrogen-bonding interactions, the endo-anomeric ($n_{O_5} \rightarrow \sigma^*_{C_1-O_1}$) effects in the α anomers, and the exo-anomeric ($n_{O_1} \rightarrow \sigma^*_{C_1-O_5}$) effects in the (t) and (g+) O_1H rotamers of the α anomer, and in the (t) and (g−) O_1H rotamers of the β anomer.

The geometrical effects of the interactions outlined above can be quantified by comparing selected conformers. Pure geometrical consequences can be derived from structures differing only in one interaction. Such is the endo-anomeric effect from **A1** and **B1**: the impact is an 0.015-Å lengthening of C_1-O_1, and an 0.009-Å shortening of C_1-O_5. The effect of the $O_{6b}-H\cdots O_5$ hydrogen bond on C_1-O_5 can be derived from the **A1**/**A3** and **A2**/**A5** pairs: it is a lengthening by 0.006−0.009 Å. The **A1**/**A2** and **B1**/**B2** pairs differ by two interactions: the break of the weak $O_1-H\cdots O_2$ hydrogen bond and the considerable exo-anomeric effect in **A2** and **B2**. The influence of the $O_1-H\cdots O_2$ hydrogen bond on C_1-O_5 is probably negligible; hence an increase by ca. 0.025 Å may be ascribed to the impact of the exo-anomeric effect.

Finally, we note some additional characteristic features found in the representative conformers of D-glucuronic acid. There is no C_1-O_1 shortening (expected from the exo-anomeric effect) in **A4**, **A10**, **B4**, **B5**, and **B11**, where a new hydrogen-bonding interaction ($O_2-H\cdots O_1$) appears. The role of O_1 as acceptor lengthens the C_1-O_1 bond considerably (especially in **A10** with the shortest hydrogen bond, cf., Figure 2 and Table 3), compensating effectively for the exo-anomeric effect. The exo-anomeric effect is weaker in **B11** (cf., Table 2), which, accompanied by the break of the $O_{6b}-H\cdots O_5$ hydrogen bond, accounts for the relatively short C_1-O_5 bond in this conformer. We observed also a slight shortening of the C_1-H bond in the conformers with pronounced exo-anomeric interactions, as reported previously for related systems.[20]

Conclusions

We performed a complete scan of the conformational space of $^4C_1$ D-glucuronic acid. Such studies are generally done by Monte Carlo or molecular dynamics methods using molecular mechanics force fields.[24] The advantages of these methods are the (relatively) low computational costs and that they can be applied for large molecules. However, the reliability of the results depends strongly on the applied force field. General force fields (MM2, AMBER, etc.) are less suitable for carbohydrates. The ones parametrized specifically for carbohydrate atoms are GROMOS[32] and CHARMM,[33] and for the treatment of solutions it is CHEAT.[34] In addition, the Monte Carlo and molecular dynamics conformational searches provide generally an incomplete set of minima on the potential energy surface. The most reliable way to locate the global and low-energy local minima is the simulated annealing approach associated with molecular dynamics.[35−37] Depending on the chosen kT parameter, however, this method will skip minima separated by energy barriers $V_{i0} < kT$.

Our approach included the generation of all of the possible rotamers varying the OH and COOH angles of torsion in glucuronic acid. The 648 initial structures for each anomer generated in this way were pre-optimized at the HF/3-21G(p) level. This level is satisfactory enough to find all of the minima on the potential energy surface. In the case of the $^4C_1$ D-glucuronic acid, our scan resulted in 58 and 62 different minima for the α and β anomers, respectively. As compared to the Monte Carlo and molecular dynamics techniques, our approach is obviously computationally more demanding; hence its applicability is limited by the size and conformational freedom of the target molecule.

More reliable geometries and relative energies of the 15 most stable conformers in each series were obtained by geometry optimizations using the B3LYP density functional theory method in conjunction with a diffuse polarized valence triple-ζ basis set. Our calculations gave a general preference of the α anomers in the isolated molecules in agreement with the endo-anomeric hyperconjugation effect. From the other intramolecular interactions (exo-anomeric hyperconjugation, hydrogen-bonding, dipole−dipole, and steric interactions), the effect of hydrogen bonding is the most pronounced and plays a major role in determining the stability order within the α and β anomers. Hence, the most stable conformers of both α and β $^4C_1$ D-glucuronic acid are the structures with the maximum number (5) of intramolecular hydrogen bonds. Introduction of solvent (water) effects by the SCI-PCM model resulted in two characteristic changes of the energy properties: the gas-phase stability order changed considerably, and the energy range of the 15 most stable conformers decreased from 30 to 15 kJ/mol.

The geometrical parameters of the conformers reflect well the superimposed effects of hyperconjugation and hydrogen-

bonding interactions. Most characteristic are the variations of the C−O bond distances (within a range of 0.04 Å) upon the combined intramolecular effects. Comparing related conformers that differ only in one interaction, we were able to determine some pure geometrical consequences. Accordingly, the impact of the endo-anomeric effect is a 0.015-Å increase of C_1-O_1, and a 0.009-Å decrease of C_1-O_5. The effects of the $O_{6b}-H\cdot\cdot\cdot O_5$ hydrogen bond and the exo-anomeric interaction on C_1-O_5 are manifested in increases by about 0.008 and 0.025 Å, respectively.

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Supporting Information Available: Cartesian coordinates of the energetically lowest 15 structures for each of the α and β conformers from the B3LYP/6-311(++)G** geometry optimizations. This material is available free of charge via the Internet at http://pubs.acs.org.

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