

A Force-Field Study of the Conformational Characteristics of the Iduronate Ring

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Received 29 April 1985; accepted 14 September 1985

Methods of molecular mechanics were applied to investigate the conformation of the (methyl 2-O-sulfate-4-methyl- α -L-idopyranose) uronic acid (DMIS), in order to correlate the peculiar vicinal proton coupling constants observed in polysaccharides containing the iduronate ring to the conformational characteristics of this sugar ring. We found three conformers with comparable energies, namely the two chair forms 1C_4 and 4C_1 and the skew-boat form ${}^2S_0(L)$; the latter is separated from each chair form by a barrier of about 9 kcal/mol. Along the pseudorotational path three additional minima (3S_1 , 1S_3 , and 1S_5) were found, yet at least 4 kcal/mol higher than 2S_0 . The results obtained for the relative energies of the three conformers and the conformation of the side groups were affected by the inclusion of the electrostatic term and, in particular, by the charge assigned to the ionic groups of DMIS. However, the conformational properties of the idopyranosidic ring in DMIS (and in related compounds) should still be interpreted in terms of equilibrium among these three conformers only.

INTRODUCTION

The ring conformation of the ido-pyranose derivatives often appears to be an unsettled problem.¹ Growing attention has been given in the recent years to the study of the conformation of L- α -iduronate, the unusual sugar found in heparin, heparan sulfate, and dermatan sulfate.²⁻⁸ In particular, it is of great interest to elucidate the ring conformation of the 2-O-sulfate- α -L-iduronate residue (hereafter indicated as I_s) belonging to the sequence of heparin pentasaccharide associated with binding to antithrombin III.⁹⁻¹¹ The pentasaccharide corresponding to such a sequence was synthesized by Sinaÿ et al.,¹² and a very recent NMR study¹³ on this compound has shown a peculiar behavior of the I_s residue, related to its conformational characteristics, thus suggesting intriguing implications with regard to the influence of the unique trisulfated D-glucosamine residue on the conformation of the adjacent I_s .

Both chair conformations ${}^1C_4(L)$ and ${}^4C_1(L)$ were considered by various authors in order to interpret experimental data on glycans containing α -L-iduronic acid;¹⁴⁻¹⁷ but in some cases also skew-boat conformations were included as possible conformers: for example, conformation ${}^1S_3(L)$ was not rejected in an

x-ray fiber diffraction study of heparin by Nieduszynski et al.¹⁸ Very recently Augé and David¹ interpreted vicinal proton-proton coupling constants of a number of idopyranoside derivatives as the indication of an equilibrium between the usual chair conformer ${}^1C_4(L)$ and the skew-boat form ${}^2S_0(L)$. The latter conformer was found in the crystalline state of methyl 4,6-O-(S)-benzylidene-2-chloro-2-deoxy- α -idopyranoside.¹⁹

Owing to the variety of proposed conformations, it may be useful to apply the current methods of molecular mechanics in order to define the regions of the conformational space energetically accessible to the idopyranose ring. In the present article we show the results of energy calculations performed on (methyl 2-O-sulfate-4-methyl- α -L-idopyranoside) uronic acid (DMIS, schematically drawn in Fig. 1). The choice of this monosaccharide derivative was mainly dictated by our interest in elucidating the conformation of I_s in the context of heparin's polymer chain. The aim of the present work was mainly to identify the ring conformers sterically allowed with a certain margin of confidence, as well as to establish the order of magnitude of the barriers hindering the conversion from one conformer to another. A more accurate analysis of the equilibria

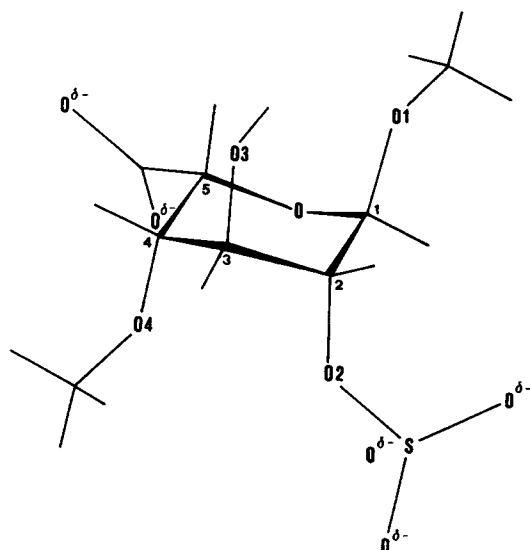


Figure 1. The monosaccharide, (methyl 2-O-sulfate-4-methyl- α -L-idopyranoside) uronic acid (DMIS) considered in this work. Torsion angles are defined as χ_1 (O—C1—O1—C), χ_2 (C1—C2—O2—S), χ_3 (C2—C3—O3—H), χ_4 (C3—C4—O4—C), χ_5 (C4—C5—C—O). Positive values of the dihedral angle correspond to clockwise rotations with respect to the syn-planar conformation, taken as zero; g^+ , g^- , and t indicate conformations with angles near 60° , -60° , and 180° , respectively.

between different conformers in various mono- and di-saccharides similar to DMIS, in connection with interpretation of proton coupling constants, will be attempted in a subsequent study.

METHOD OF CALCULATION

Growing attention has been paid in the last years to the application of the molecular mechanics approach to the study of carbohydrate conformation. The so-called HSEA (hard-spheres exo-anomeric effect) method developed by Lemieux and associates,^{20,21} is quite popular for its computer time economy and its proven usefulness in connection with ^1H NMR studies.^{22,23} This method, however, as it considers the sugar ring moiety as a rigid entity, thus defining the conformational energy as a function of the ϕ and ψ rotation angles around the glycosidic bonds, is not suitable for examining the flexibility of the iduronate ring in the heparin chain. Other force fields, more complete inasmuch as they include both bonded and nonbonded interactions and allow the release of all molecular degrees of freedom, are available in the literature. Some such force fields were designed

to deal with carbohydrates: Rasmussen and coworkers applied their consistent force field²⁴ to determine the conformational structure of various disaccharides;^{25,26} Jeffrey and Taylor²⁷ modified some parameters of Allinger's MMI program²⁸ in order to best fit the geometries of pyranose and methyl pyranoside molecules; Tvaroška²⁹ accounted for solvent effects on the relative stability of saccharide conformers by using the continuum reaction field method. No force field, however, was readily suitable for application to heparin's ionic and sulfated constituents. Therefore, we decided to utilize Allinger's well known MM2 program³⁰ as the starting point for building a force field which could deal with heparin-like molecules.

The program MM2 calculates (and minimizes) the molecular energy as the sum of stretching, bending, stretch/bend, torsion, Van der Waals, and Coulomb terms: except for the modifications stated below, the original MM2 parameters³⁰ were utilized as input to program REFINE/U in use in our laboratory.³¹

As the latter program adopts the monopole approximation for computing the Coulomb energy, the partial charges on most atoms were derived from the MM2 bond-dipole moments: an effective value of 3 was taken for the dielectric constant. The charge on the carboxylate and sulfate ions was distributed according to the following rather crude approximation: taken as q electrons the total ionic charge (with $q < 1$ to simulate the screening due to counterions), $0.3q$ was assigned to the three equivalent $\text{O}^{\delta-}$ atoms of the sulfate group and $0.1q$ to the oxygen bonded to the ring; similarly $0.4q$ and $0.2q$ were assigned, respectively, to $\text{O}^{\delta-}$ and C of the carboxylate ion. Standard -O- Van der Waals type (type No. 6 in ref. 30, bearing lone pairs) was assigned to all oxygens connected to the ring, including the one bonded to S, while Van der Waals type $\text{O}=\text{}$ (i.e., type 7, without lone pair pseudoatoms) was assigned to the $-\text{CO}_2^-$ and the $-\text{SO}_3^-$ oxygens. Zero torsional term was assumed for rotations around bonds $\text{C}-\text{C}-\text{CO}_2^-$, $\text{C}-\text{C}-\text{SO}$, and $\text{O}-\text{C}-\text{SO}_3^-$.

In order to account for hydrogen bonding we had to modify some of the original Van der Waals parameters concerning the potential donors and acceptors of a hydrogen bond $\text{O}-\text{H}\cdots\text{O}$. The data utilized for this

purpose were the O...O equilibrium separation observed in ice and the interaction energies of various water dimer arrangements calculated by Matsuoka, Clementi, and Yoshimine³⁴ with the configuration-interaction method. These energies were recently utilized by Kroon-Batenburg and Kanters³⁵ in order to develop an empirical O—H...O hydrogen bond potential compatible with the MM2 force field; however, such a potential did not appear readily applicable to our program. Hence we decided to approximately fit the above-mentioned data just by varying the parameters for the interactions between the acceptor oxygen and the hydrogen (the chosen values are $r^* = 2.3$ Å, $\epsilon^* = 0.04$ kcal/mol) and between the lone pair and the hydrogen ($r^* = 1.6$ Å, $\epsilon^* = 0.048$ kcal/mol).³⁶

The stretching and bending parameters of the carboxylate group were taken from previous calculations: $l^\circ(\text{C}—\text{C}) = 1.52$ Å, $l^\circ(\text{C}—\text{O}) = 1.25$ Å, $\theta^\circ(\text{O}—\text{C}—\text{O}) = 120^\circ$. As for the sulfate group, the following geometrical parameters, derived from the analysis of the data provided by the crystal structures of a number of model compounds³⁸ were used: $l^\circ(\text{O}—\text{S}) = 1.60$ Å, $l^\circ(\text{S}—\text{O}^-) = 1.42$ Å, $\theta^\circ(\text{C}—\text{O}—\text{S}) = 110^\circ$, $\theta^\circ(\text{O}—\text{S}—\text{O}^-) = 102^\circ$, $\theta^\circ(\text{O}^{\delta-}—\text{S}—\text{O}^{\delta-}) = 115^\circ$; force constants of 3 mdyne/Å for the stretchings and 0.3 mdyne Å/rad² for the bendings were assumed.

RESULTS

The gross features of the conformational energy surface of a ring molecule such as DMIS, carrying several bulky substituents,

should result from the balance between strain energies (ring closure, bending, and torsional terms, including also the anomeric effect) and steric interactions. Other energy terms, such as electrostatic interactions and solvent effects, although very important in a polar molecule, are expected to show a smoother behavior, so that inclusion of these terms in the calculation may alter the relative stability of the energy minima, even quite significantly, but it can hardly yield stable conformers in regions severely forbidden by the former type of forces. Therefore, we set out to explore the energy surface of DMIS by omitting the electrostatic term from the force field previously described.

In a first step we searched for low-energy ring conformations starting from various standard forms (chair, boat, skew-boat). Only three conformers with comparable energies were found, namely the two chair forms ${}^1\text{C}_4$ and ${}^4\text{C}_1$, and the skew-boat form ${}^2\text{S}_0$. For each of these three conformers we proceeded to rotate the five side-chains, in order to determine all the local minima: for every new minimum found, the total molecular energy was minimized without any restriction. This led to the energy minima listed in Table I. It was found that, for all three ring forms, only one conformation of the glycosidic bond has low energy, namely that favored by the exo-anomeric effect. Rotation of the sulfate group shows only one minimum, corresponding to a nearly *cis* conformation of H2 with respect to the sulfur atom; however changes of χ_2 by $\pm 15^\circ$ can occur with an energy increase of ~ 2 kcal/mol. A rather wide range is allowed for the rotation of the carboxylate group: when the electrostatic term is omitted, this

Table I. Main geometrical features of the minimum-energy structures.^a

Conformation	θ	ϕ_2	Q	χ_1	χ_2	χ_3	χ_4	χ_5	E _{total}
${}^1\text{C}_4$	5	238	0.56	-69	107	-67	74	-98	0.06
	4	245	0.56	-68	107	-170	73	-98	0.0 ^b
	3	258	0.56	-68	107	50	74	-99	0.73
${}^2\text{S}_0$	89	265	0.77	-70	114	-71	67	-104	0.80 ^b
	89	262	0.76	-70	118	-175	68	-103	1.33
	89	265	0.77	-70	116	57	68	-104	1.51
	176	177	0.58	-74	118	-70	172	-5	0.21 ^b
${}^4\text{C}_1$	176	173	0.58	-74	120	-172	172	-6	0.23
	176	176	0.58	-73	119	60	173	-6	0.90
	177	179	0.58	-74	118	-68	101	-1	1.28

^aParameters θ , ϕ_2 and Q are defined in Ref. 39 (see also text).

^bThe corresponding molecular models are shown in Figure 2.

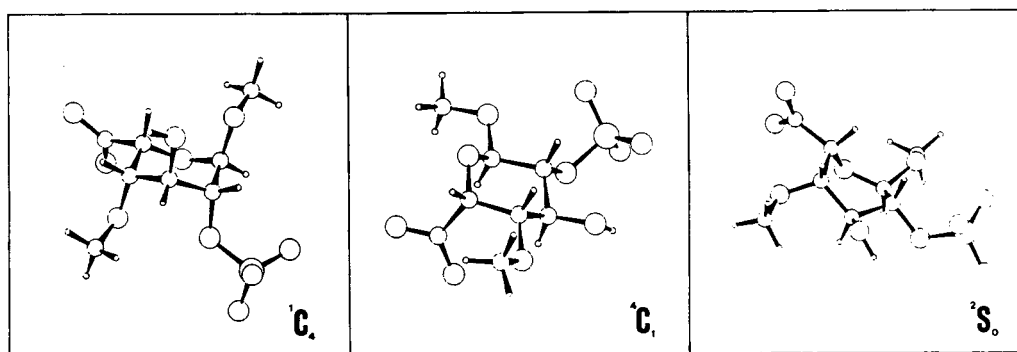


Figure 2. The three energetically most favored arrangements of DMIS (see also Table I).

rotation follows up that of other side-chains at a small energy cost. Also rotation around bond C4—O4 shows only one stable conformer, except for the 4C_1 form, which has a secondary minimum at $\chi_4 \sim 100^\circ$, about 1 kcal/mol above the t conformer. *Vice versa*, the hydroxyl group is the only one allowed to take all three staggered conformations; their relative stability will largely depend on the electrostatic interactions and/or the formation of hydrogen bonds. Figure 2 shows the molecular models for the most favored arrangements of the three ring conformers; the corresponding geometrical features are given in Table I.

Secondly, we computed the minimum energy paths connecting the stable conformers, in order to estimate the barriers hindering conversion from one conformer to another. Here we describe the ring conformation by means of the ring puckering variables defined by Cremer and Pople.³⁹ For the pyranosidic

ring there are three such variables, namely q_3 (representing the amplitude of the chair type puckering) and the amplitude-phase pair (q_2, ϕ_2), representing the boat/twist-boat type of puckering. By taking $q_2 = Q \sin \theta$, $q_3 = Q \cos \theta$, the above three variables can be replaced by the spherical polar set (Q, θ, ϕ_2), which allows the mapping of various pyranose ring conformations on a spherical surface. In this representation two of the three energy minima lie near the two opposite poles (1C_4 and 4C_1), while the third one (2S_0) lies on the equator. Thus we chose θ as the "driving" variable⁴⁰ for computing the path connecting 2S_0 to the two chair forms. In these calculations the molecular energy is minimized while constraining the driving variable to a given value, and this value is changed by steps (5°) to draw the path. Similarly, we took the variable ϕ_2 for computing the minimum energy profile along the pseudorotational path near the equator, starting

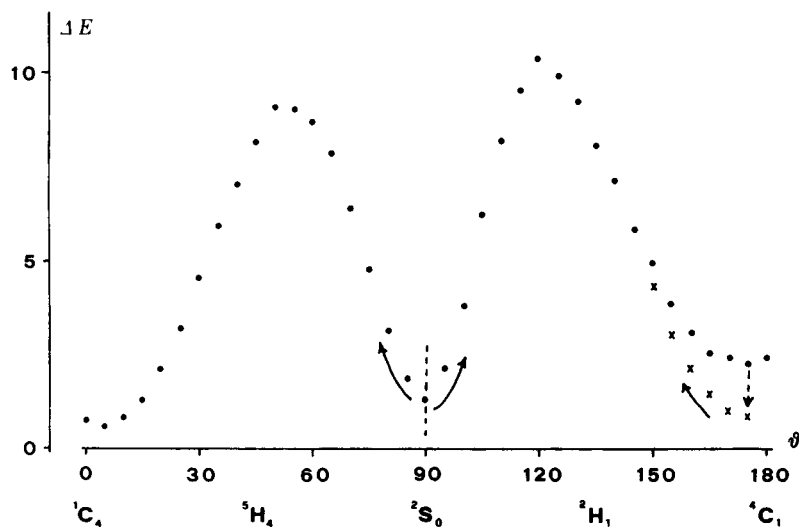


Figure 3. Energy profile along the path (where θ is driven) from the ring conformation 2S_0 to 1C_4 and 4C_1 , respectively. Crosses represent part of the path from a deeper 4C_1 minimum (with $\chi_4 \sim t$).

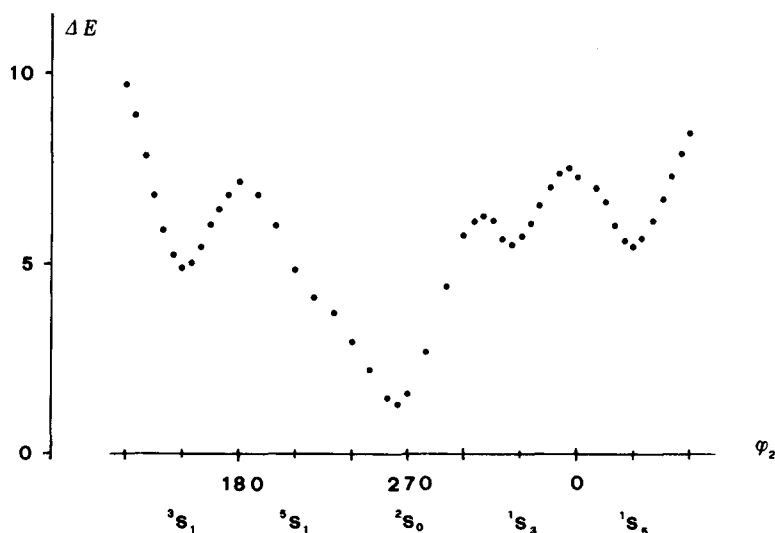


Figure 4. Energy profile along the pseudorotational path (where ϕ_2 is driven) from the ring conformation 2S_0 to 3,0B and to $B_{2,5}$ respectively.

from 2S_0 . The results are shown in Figures 3 and 4. Finally, the "flexibility" of each of the three conformers was estimated by computing the two-dimensional energy map $E(\phi_2, \theta)$ (Fig. 5), using the same constrained energy minimization technique.⁴¹

The various minimum energy conformers, obtained by assuming $\epsilon = \infty$ in the computations just described, were then used as starting points for new energy minimizations which included the electrostatic term. For these computations the effective value of the dielectric constant was taken as 3; moreover

the total charge q on the ionic groups was varied from 0 to 0.5 electrons. The results of these calculations are shown in Figure 6.

DISCUSSION

A first glance at the map of Figure 5 indicates that the conformational energy surface of DMIS broadly resembles that of the simplest parent compound, namely cyclohexane. It is well known⁴² that in cyclohexane the two equivalent stable chair forms can convert into each other by going over a barrier of ~ 11 kcal/

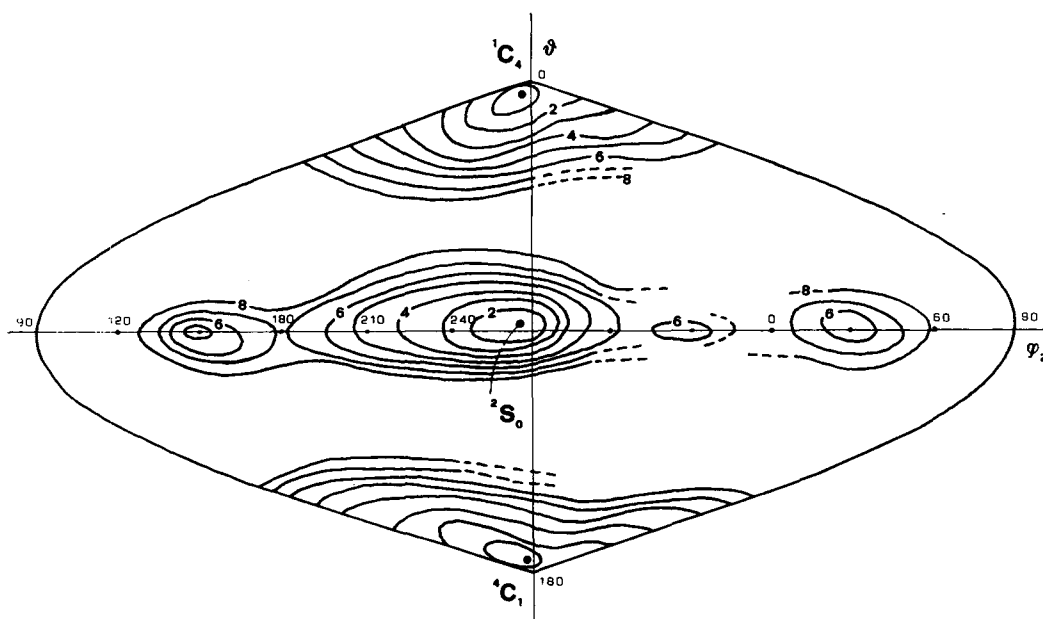


Figure 5. Equivalent projection of the isoenergetic contours on the spherical (ϕ_2, θ) surface, taking $\phi_2 = 270^\circ$ as central meridian. Energy levels (referred to the 1C_4 minimum) differ by 1 kcal/mol; minima near 1C_1 and 2S_0 are at 0.21 and 0.80 kcal/mol, respectively.

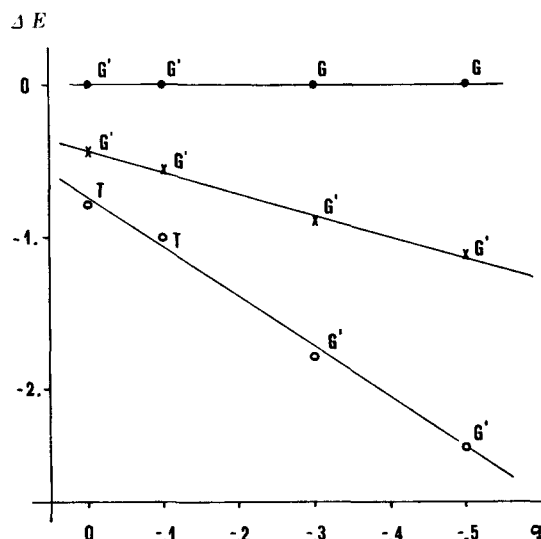


Figure 6. Effect of the inclusion of the electrostatic term on the energy difference among the three more stable ring conformers: q is the charge (in electrons) on the ionic side-groups. ΔE (in kcal/mol) for 4C_1 (○) and 2S_0 (×) are referred to the 1C_4 (●) energies. The approximate conformation of χ_3 is indicated.

mol⁴³ (corresponding to the half-chair form) and passing through local minima (the skew-boat forms) higher by ~ 5.5 kcal (by symmetry there are six equivalent skew-boats separated by as many barriers of ~ 1 kcal/mol corresponding to the boat forms). Incidentally, we observe that the MM2 force field³⁰ reproduces the features of cyclohexane with good approximation. Also in DMIS there are two nearly equienergetic chair forms, separated by two high-energy regions and by an intermediate equatorial zone. However, inspection of Figure 3 shows that the path connecting the two chair forms passes through a unique intermediate skew conformer (2S_0), which is much more stable (ΔE only 0.8 kcal/mol) in comparison with the cyclohexane case. The stabilization of 2S_0 in DMIS arises from the equatorial or synclinal orientations of the side groups. On the contrary, the usual 1C_4 chair is destabilized by having four axial substituents, while the 4C_1 chair is unfavored by the strong axial effect of the carboxylate group, as well as by the anomeric effect. For both chair forms the destabilization is such as to roughly balance the intrinsic strain of the skew-boat ring conformation observed in cyclohexane.

The heights of the barriers separating 2S_0 from the two chair forms are slightly lower than the barrier observed in cyclohexane (8.1 and 9.7 kcal/mol, respectively, for 1C_4 and

4C_1). Figures 3 and 5 also show that 2S_0 is a conformer as well defined as the rigid chair forms: indeed the areas of the regions within a given level, say 1 kcal/mol, above each minimum are approximately equal.

The 2S_0 conformer is the only skew-boat form being favored by the absence of axial interactions. Figures 4 and 5 show three more energy minima (3S_1 , 1S_3 , and 1S_5) which are less stable by about 4 kcal/mol and, therefore, are unlikely to play a significant role in the conformational equilibrium, even when other forces may be present.

Introduction of the electrostatic terms favors the conformers 2S_0 and 4C_1 with respect to 1C_4 . Figure 6 shows that when the charge on the two ionic groups is assumed to be completely screened ($q = 0$) the three conformers are almost equienergetic, while the ionic term mainly stabilizes the 4C_1 conformer, due to a favorable interaction of the sulfate group with the hydroxyl group in the g^- conformation ($\chi_3 \sim -60^\circ$).

Although the present calculations do not allow us to assess the conformer populations due to the many approximations involved in the force field, we may conclude that the conformational properties of the idopyranosidic ring in DMIS (and in related compounds) should be described in terms of equilibrium among three conformers. We are confident that the growing wealth of data concerning the proton-proton coupling constants of various iduronic acid derivatives can be interpreted by means of such conformational equilibrium. This in turn may be of help in order to refine the present methods of calculation. For this purpose work is in progress in our laboratory.

Augé and David¹ already pointed out the need to consider the 2S_0 conformation for explaining some unusual coupling constants in idose derivatives. They also associated the variability of the observed coupling constants with the flexibility of the twisted-boat form. Our calculations rather suggest that also 2S_0 is a well-defined conformer, so that changes in the coupling constants should correspond to changes in the equilibrium among conformers. Such an indication appears in Figure 7 where we report the energy profile for the conversion from 1C_4 to 2S_0 computed by taking the torsional angle H-C2-C3-H as the "driving" variable. The figure shows also

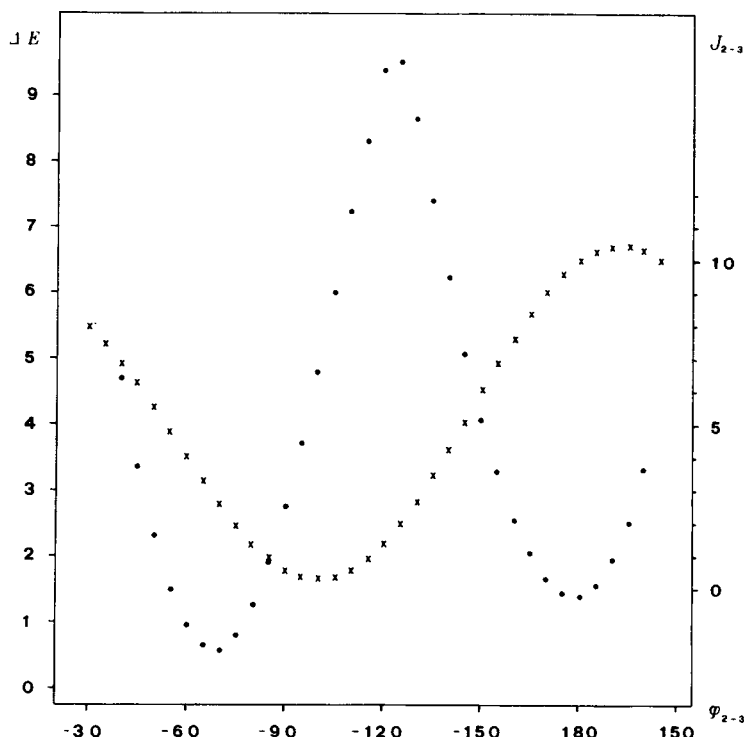


Figure 7. The proton-proton coupling constant J_{2-3} computed according to ref. 44 (x) and the energy (●) vs. ϕ_{2-3} (H—C2—C3—H), while this angle is driven in order to convert the ring form from 1C_4 to 2S_0 .

the plot of the coupling constant J_{2-3} calculated according to the equation proposed by Haasnoot, deLeeuw, and Altona.⁴⁴ While this curve shows a small J_{2-3} value for the 1C_4 energy minimum and a large one for 2S_0 , the shape of the energy barrier seems to rule out that the observed values of J_{2-3} , in the range 4.5–7.5,^{5, 7, 13, 45} can be attributed to a single deformed conformer.

The authors are grateful to B. Casu, G. Gatti, J. Choay, and their coworkers for stimulating discussions.

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41. It is clear that, in order to get an accurate description of the energy surface, other degrees of freedom should be taken into account, e.g., those related to the side-chain conformation. Burkert and Allinger have warned against deficiencies inherent to such methods of computing energy profiles and maps. As each point represents the minimum with respect to many variables, errors may arise not only from lack of convergence, but also from the inability to overcome energy barriers in the multidimensional space. The path connecting 2S_0 to 4C_1 provides an example of such problems, as the torsional angle χ_4 (C3—C4—O4—C) presents two distinct conformations for the two ring conformers. When starting from 2S_0 , the χ_4 conformation remains *g* also beyond the barrier and even at the 4C_1 minimum of the profile ($\theta = 175^\circ$, $\chi_4 = 71^\circ$), which is ~ 0.5 kcal/mol higher than the minimum ($\chi_4 = 100^\circ$) listed in Table I. By taking χ_4 as the driving variable, one finds that energy slowly decreases from *g* to a minimum at $\chi_4 = 100^\circ$, then, after a 0.5 kcal/mol barrier around 130° , drops to the *t* minimum. When the θ profile is computed from 4C_1 to 2S_0 , a barrier higher by ~ 1.2 kcal/mol is found at $\theta = 120^\circ$, as the *t* value of χ_4 is severely unfavored for the skew-boat form. Clearly, in this case a thorough analysis would require the calculation of a two-dimensional map $E(\theta, \chi_4)$. These arguments, however, do not affect our conclusions concerning the position and the shape of the energy minima nor the heights of the barriers.
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