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CONFORMATIONAL ANALYSIS OF GLYCOSAMINOGLYCANS. I. CHARGE DISTRIBUTIONS, TORSIONAL POTENTIALS, AND STERIC MAPS

RUDOLPH POTENZONE, JR. AND ANTON J. HOPFINGER*

Department of Macromolecular Science, Case Western Reserve University, Cleveland, Ohio 44106 (U. S. A.)

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ABSTRACT

An initial study of the charge distributions and torsional potentials for (1→4)-linked disaccharides, and a steric energy-map for (1→4)-linked disaccharides is reported for six acidic glycosaminoglycans. The charge distributions have been computed by the CNDO quantum-mechanical procedure, together with a molecular-decomposition technique. The intrinsic torsional potential has been computed by using CNDO energies and empirical potential-energies. The intrinsic torsional potential is two-dimensional. The general steric-map for a (1→4)-linked disaccharide will be useful for simplifying future conformational analyses of glycosaminoglycans. Wherever possible, comparisons between theory and experiment are presented or cited.

INTRODUCTION

This is the first in a series of papers concerned with the conformational analysis of connective-tissue glycosaminoglycans (**1**). The impetus for this research comes from a joint investigation with Blackwell and coworkers, who have carried out extensive studies[1–9] of dilute solutions containing **1** and poly-α-amino acids, which are considered to be models for connective-tissue structure. In connective tissue, collagen, having a relatively high content of positively charged amino acid residues (lysine and arginine) is usually found embedded in an acidic matrix of **1**. These acidic **1** are found to possess a net negative charge and are generally sulfated to various degrees. Blackwell and coworkers have been able to identify structure-stabilizing factors that may be relevant to the structural organization of the collagen–**1** matrix.

Previous work, both experimental and theoretical, on the conformational properties of **1** has been very limited. The chemistry and structural repeating units of **1** have been reviewed[10], and Atkins and coworkers have reported work on the geometry of **1** by X-ray crystallography[11–14]. The crystal structure of chondroitin 6-sulfate has

*To whom requests for reprints should be sent.

also been studied[15]. There have been some studies aimed at elucidating the structural properties of **1** in solution[16,17]. Theoretical conformational analysis of **1** has been limited to determining the sterically allowed conformations of the disaccharide residues[18]. One investigation has been concerned with the conformation of hyaluronic acid by using Van der Waals' interactions and a torsional potential[17]. There has been some work, by different groups, using empirical potential-energy functions, on the theoretical conformational analysis of polysaccharides[17–27]. Molecular-orbital calculations on disaccharide subunits[28] have also been attempted.

Our group has carried out conformational analyses of polypeptides, drug molecules, and, to a lesser extent, various synthetic polymers[29–34]. Ref. 35 discusses in detail the empirical potential-energy functions used in calculations of this type. An added dimension in these analyses is the inclusion of solvent effects upon molecular conformation[35]

In this initial study of the structure of **1**, we report partial charge-distributions on each (1→4)-linked disaccharide, and the intrinsic, two-dimensional, torsional potential for rotation about the (1→4) linkage. These calculations are in preparation for computing the conformational properties, in a vacuum and in dilute aqueous solution, of six acidic **1**, namely, hyaluronic acid, chondroitin, chondroitin 4-sulfate, chondroitin 6-sulfate, dermatan sulfate, and keratan sulfate. Heparin, having at least a tetrasaccharide repeating unit, will not be considered in this initial report. Wherever possible, a comparison between theory and experiment is made. In some instances experiments are suggested.

The ultimate goal of these studies is to consider, on a purely theoretical basis, possible "complex" formation between **1** and charged poly-α-amino acids that should help to explain the structural organization of connective tissue.

DISCUSSION

General aspects of conformational analysis of glycosaminoglycans (**1**). — Several properties related to primary structure need to be considered in the conformational analysis of **1**. First, it is to be noted that these biopolymers have a disaccharide repeating unit. In these studies, the structural monomer unit is defined as the two different (1→4)-linked monosaccharides. These structural monomer units are then (1→3)-linked to form the primary structure of the biopolymer. These studies are concerned initially with the distribution of conformational states for each of the (1→4)-linked disaccharide units. Later, two (1→4)-linked disaccharides, themselves (1→3)-linked forming a tetrasaccharide, will be subjected to these conformational analyses. The general disaccharide units are shown in Fig. 1.

The conformation of a disaccharide unit is defined in accord with the IUPAC convention. The rotations are defined about the C–O–C linkage as θ_1 and θ_2, as shown in Fig. 2. Residue 1 is always 2-acetamido-2-deoxy-D-galactose, and unit 2 is the D-glucuronic acid residue, except for keratan sulfate, where the order is reversed. The angle θ_1 is defined as 0° when H-1 is *cis* to C-4′ about the C-1–O-1 bond (see Fig. 2).

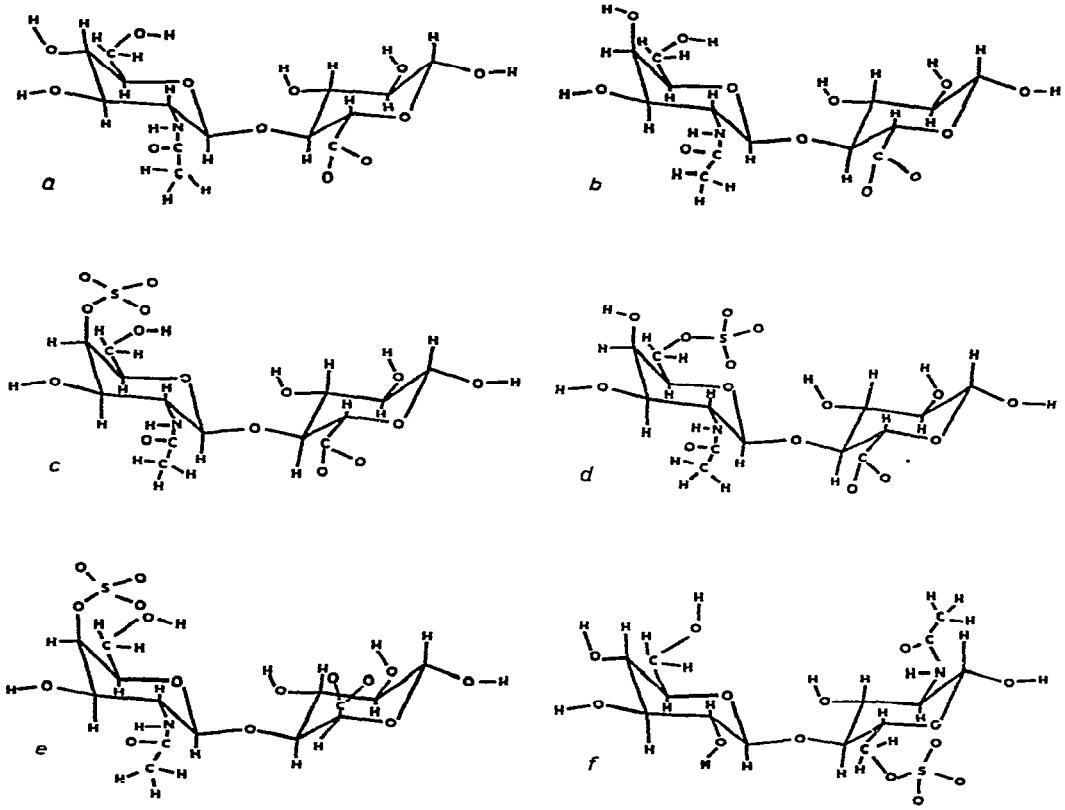


Fig. 1. The structure of the structural monomer [(1→4)-linked disaccharide] of the six glycosaminoglycans; (*a*) hyaluronic acid, (*b*) chondroitin, (*c*) chondroitin 4-sulfate, (*d*) chondroitin 6-sulfate, (*e*) dermatan sulfate, and (*f*) keratan sulfate.

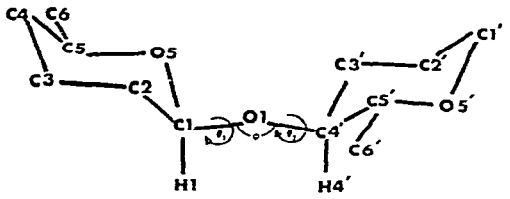


Fig. 2. General disaccharide unit, showing the (1→4) linkage with θ_1 and θ_2 indicated. H-1 is *cis* to C-4′. C-1 is *cis* to H-4 . The bond angle, $\varnothing$, is also indicated.

Clockwise rotation is positive. The angle θ_2 is 0° when C-1 is *cis* to H-4′ about the O-1–C-4′ bond. The coordinates for the backbone atoms of each disaccharide are derived from X-ray data of D-xylan, as reported for chondroitin 6-sulfate[15]. Modifications of this molecule were made to form the various monosaccharides of **1** by using standard bond-distances and angles, as given in ref. 35.

The more-striking features of **1** from a conformational point of view are: (*a*) the marked steric restrictions to rotations about θ_1 and θ_2 arising from the bulky hexose rings, and (*b*) the net -1 e.u. and -2 e.u. charges of each disaccharide. These factors play an important role in determining the distribution of conformational states in these biopolymers. Rotations about θ_1 and θ_2 involve an intrinsic, orbital-deformation, torsional-energy contribution, which has been determined as part of this study. The bond angle ϕ formed by the C–O–C linkage has been discussed by several workers[19–21]. The geometry used here has $\phi = 117.35°$. The conformational consequences of varying this bond angle will be considered in future studies when a relationship between bond angle and bond-angle energy has been established.

Atomic-charge calculation for large molecules. — Previous charge-distribution calculations on structural units of polysaccharides have been limited to the Del Re method for the σ-charge contribution, and simple Hückel theory for the π charges[21,22,25]. Recently, charge distributions on some carbohydrate molecules were determined by the molecular-orbital CNDO/2 method[26]. This molecular-orbital treatment is particularly well suited for calculating charge distributions on small molecules, especially those composed of atoms of the second period[36]. CNDO/2 yields partial-charge distributions as a function of conformation, and not just primary structure. as is the case with both the Del Re and simple Hückel methods. A comparison of various methods to compute charge distributions for formamide and some model peptides favor the CNDO/2 method as being best in reproducing experimental quantities such as dipole moments[37]. We have adopted the CNDO/2 scheme in this work on **1**. However, computer space and computation time critically limit the size of the molecules that can be considered in these analyses. A maximum of 80 orbitals per molecule can be handled with the version of CNDO used[36]. Generally, this corresponds to approximately 30 atoms. It has thus been found necessary to develop a means of decomposing the complete disaccharide units (~ 50 atoms) into smaller, calculable subunits.

The first step in such a molecular decomposition is to determine the various structural subunits that contain fewer than 80 orbitals. In order to include all of the atoms that meaningfully contribute to the charge of a particular atom or group of atoms, the subunits must include (*a*) all atoms bonded to the subunit, and (*b*) those non-bonded atoms that are "near" to the subunit and contribute to the electron density of the subunit. In the molecular-decomposition scheme used, these non-bonded species include bonded atoms up to third nearest-neighbor. Once all of the necessary atoms have been identified, the orbitals on the "end atoms" of the decomposed subunit are saturated with methyl groups of the atom next to the "end atom", if this is a carbon atom. If the atom next to the "end atom" is oxygen, a hydrogen atom is added. This unit must now be checked to ensure that it contains no more than 80 orbitals. If it does not, the charge distribution of the subunit can be determined from the electron densities as calculated with CNDO. The resulting charges on the initial portions of each subunit are assigned to the respective atoms in the original molecule. It may be necessary to adjust atomic charges on atoms that

end up as part of more than one subunit. The agreement of the charges on each of these "multiple-subunit" atoms is an indication of the reasonableness of the decomposition scheme with respect to the distribution of electron density in a given molecule.

For the disaccharide components of **1**, the foregoing general technique can be made more specific. The individual monosaccharide units are first decomposed. Each decomposition necessarily includes at least one charge calculation for a hexose ring, with the necessary side-groups in sterically-allowed orientations. The entire monosaccharide is then recomposed and the total charge is evaluated. The linkage oxygen-atom is left off at this point for each monosaccharide. The charge of this oxygen atom is determined by adding the net charges of the two monosaccharide units and assigning the residual charge of the total molecule to the oxygen atom.

Sample charge-calculation and results. — The charge calculation for the repeating unit of chondroitin 6-sulfate by the decomposition technique is presented here for illustrative purposes. Considering the 80 orbital limit, this molecule can be thought of as four distinct components. The D-glucuronic acid residue is directly

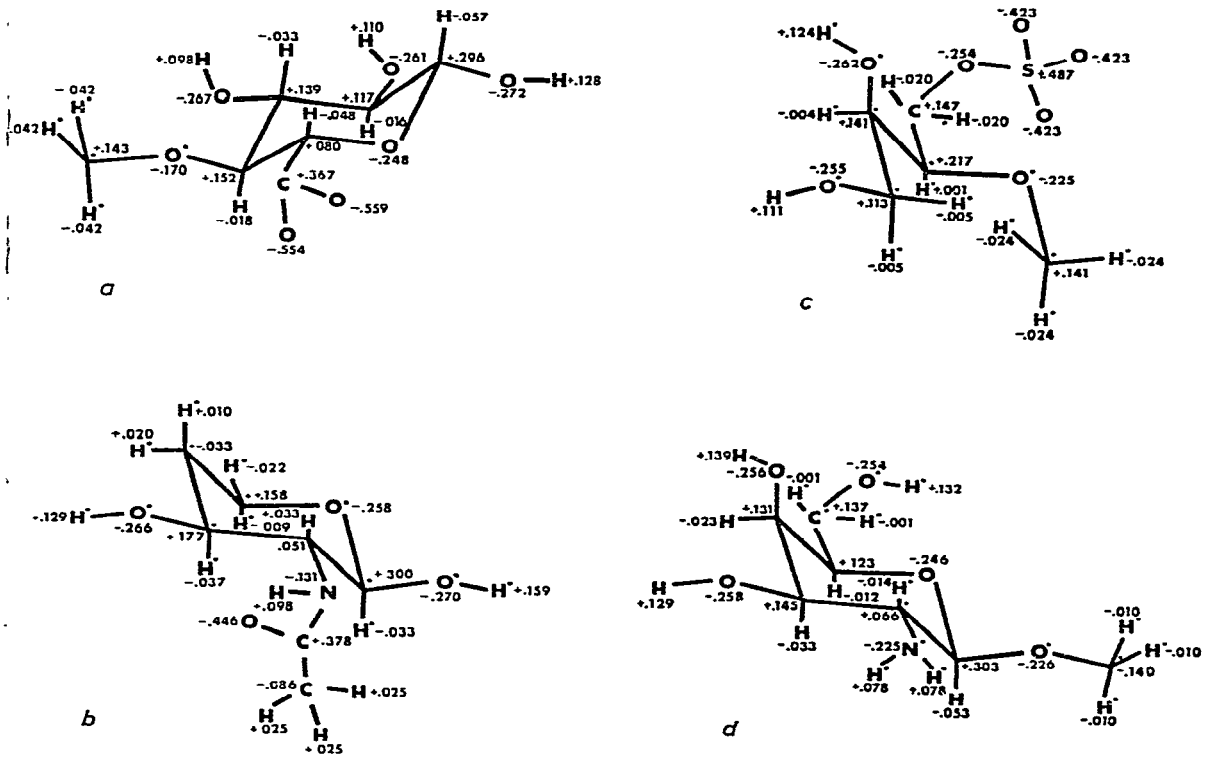


Fig. 3. The four subunits into which the chondroitin 6-sulfate repeating-unit is decomposed to calculate the charge. Those atoms without the asterisk are the actual atoms of the subunits. Those with the asterisk are those added (see text): (*a*) D-glucuronic acid subunit; (*b*) acetamido subunit; (*c*) 6-sulfate subunit; and (*d*) 2-amino-2-deoxy-D-galactose subunit.

calculable. This subunit retains the bridge oxygen atom, which is methylated in the model subunit (see Fig. 3a). For the 2-acetamido-2-deoxy-D-galactose 6-sulfate residue, two large groups are present on the hexose ring, namely the sulfate and the acetamido groups. The hexose-ring subunit is shown in Fig. 3d. Figs. 3b and 3c show the two remaining subunits with the necessary additional atoms. Those marked with an asterisk are the atoms added to the basic subunit to give the necessary contributions to the electron densities. Only the charges on the atoms of the *basic subunit* are assigned to the corresponding atoms in the actual molecule. Charges on equivalent hydrogen atoms, such as on a methyl group, are averaged.

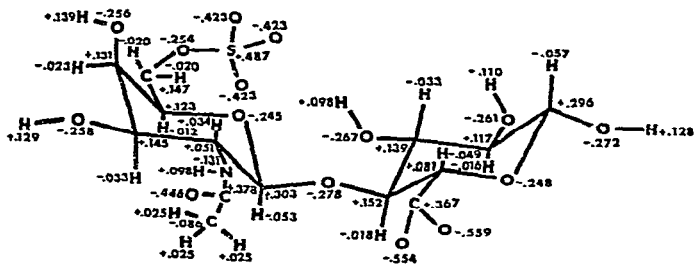


Fig. 4. Reassembled subunits of Fig. 3 forming chondroitin 6-sulfate, showing the atomic charges. Net molecular charge, $C = -2.0000$.

Fig. 4 shows the total structure of chondroitin 6-sulfate with the final charge-distribution. Table I gives the atomic charges of **1** calculated by the decomposition technique. Of particular note is the observation that respective partial-charges assigned to the recomposed molecule are virtually identical to those reported to be in agreement with experimental observations[26]. We are aware that the technique must be used carefully and with judicious selection of the decomposition subunits. However, the decomposition technique as applied to **1** has a built-in check to warn the user of errors. The oxygen atom of the acetal linkage is not calculated by CNDO, but instead is assigned a residual charge so as to produce a pre-chosen, total, net charge on the molecule. If a separate CNDO calculation is performed wherein the partial charge of the oxygen atom is computed directly, a comparison of these two partial charges on oxygen atoms should be a measure of the reliability of the decomposition. Table II lists the atomic charges for the six oxygen atoms of the linkages in molecules of **1** These partial charges are very similar to those resulting from the more-conventional calculations[26]. We consider that this is reasonable evidence that the decomposition technique gives atomic charges that can be used with confidence for theoretical conformational analysis of **1**.

A comparison with experimental dipole-moment data would have given further verification of the procedure. Dipole moments have been reported for arabinose, xylose, glucose, fructose, galactose, mannose[38], and sucrose[39], but none have been described for **1**. Comparison of dipole moments calculated by using CNDO electronic

TABLE I

ATOMIC CHARGES FOR THE ATOMS OF THE SIX DISACCHARIDE UNITS OF GLYCOSAMINOGLYCANS (1)[a]

Atom	*Hyaluronic acid*	*Chondroitin*	*Chondroitin 4-sulfate*	*Chondroitin 6-sulfate*	*Dermatan sulfate*	*Keratan sulfate*
1-linked hexose ring						
C-1	0.303	0.303	0.303	0.303	0.303	0.302
H-1	−0.049	−0.053	−0.053	−0.053	−0.053	−0.047
C-2	0.051	0.051	0.051	0.051	0.051	0.114
H-2	0.034	0.034	0.034	0.034	0.034	−0.009
O-2						−0.250
HO-2						0.130
C-3	0.150	0.145	0.159	0.145	0.159	0.146
H-3	−0.024	−0.033	−0.057	−0.033	−0.057	−0.021
O-3	−0.257	−0.258	−0.223	−0.258	−0.223	−0.259
HO-3	0.130	0.129	0.104	0.129	0.104	0.123
C-4	0.129	0.131	0.143	0.131	0.143	0.136
H-4	−0.012	−0.023	−0.014	−0.023	−0.014	−0.009
O-4	−0.258	−0.256	−0.259	−0.256	−0.259	−0.254
HO-4	0.132	0.139		0.139		0.134
C-5	0.114	0.123	0.129	0.123	0.129	0.127
H-5	−0.008	−0.012	−0.036	−0.012	−0.036	−0.014
O-5	−0.252	−0.245	−0.245	−0.245	−0.245	−0.260
C-6	0.137	0.137	0.132	0.147	0.132	0.144
H-6	−0.005	0.000	0.004	−0.020	0.004	−0.034
H-6	−0.005	0.000	0.004	−0.020	0.004	−0.034
O-6	−0.253	−0.254	−0.269	−0.254	−0.269	−0.256
HO-6	0.135	0.132	0.121		0.121	0.140
4-linked hexose ring						
C-1′	0.296	0.296	0.296	0.296	0.266	0.299
H-1′	−0.057	−0.057	−0.057	−0.057	0.039	−0.034
O-1′	−0.272	−0.272	−0.272	−0.272	−0.283	−0.270
HO-1′	0.128	0.128	0.128	0.128	0.115	0.154
C-2′	0.117	0.117	0.117	0.117	0.127	0.051
H-2′	−0.016	−0.016	−0.016	−0.016	−0.035	−0.009
O-2′	−0.261	−0.261	−0.261	−0.261	−0.259	
HO-2′	0.110	0.110	0.110	0.110	0.104	
C-3′	0.139	0.139	0.139	0.139	0.144	0.145
H-3′	−0.033	−0.033	−0.033	−0.033	−0.005	−0.034
O-3′	−0.267	−0.267	−0.267	−0.267	−0.272	−0.258
HO-3′	0.098	0.098	0.098	0.098	0.097	0.127
C-4′	0.152	0.152	0.152	0.152	0.165	0.129
H-4′	−0.018	−0.018	−0.018	−0.018	−0.057	−0.024
C-5′	0.081	0.081	0.081	0.081	0.067	0.120
H-5′	−0.048	−0.048	−0.048	−0.048	−0.033	−0.013
O-5′	−0.248	−0.248	−0.248	−0.248	−0.266	−0.247
C-6′	0.367	0.367	0.367	0.367	0.362	0.147
H-6′						−0.020
H-6′						−0.020
O-6′	−0.559	−0.559	−0.559	−0.559	−0.555	−0.254
O-6′	−0.554	−0.554	−0.554	−0.554	−0.570	

(*Table continued on next page*)

TABLE 1 (*continued*)

Atom	*Hyaluronic acid*	*Chondroitin*	*Chondroitin 4-sulfate*	*Chondroitin 6-sulfate*	*Dermatan sulfate*	*Keratan sulfate*
Sulfate						
S			0.491	0.487	0.491	0.487
O			−0.422	−0.423	−0.422	−0.422
O			−0.422	−0.423	−0.422	−0.422
O			−0.422	−0.423	−0.422	−0.422
Acetamido group						
N-2 (N-2′)	−0.131	−0.131	−0.131	−0.131	−0.131	−0.131
HN-2	0.098	0.098	0.098	0.098	0.098	0.098
C-7 (C-7′)	0.378	0.378	0.378	0.378	0.378	0.378
O-7 (O-7′)	−0.446	−0.446	−0.446	−0.446	−0.446	−0.446
C-8 (C-8′)	−0.086	−0.086	−0.086	−0.086	−0.086	−0.086
H-8 (H-8′)	0.025	0.025	0.025	0.025	0.025	0.025
H-8 (H-8′)	0.025	0.025	0.025	0.025	0.025	0.025
H-8 (H-8′)	0.025	0.025	0.025	0.025	0.025	0.025

[a]Charges are given in electronic units. For the charges on the linkage oxygen atoms and net charge, see Table II.

TABLE II

NET MOLECULAR CHARGES AND PARTIAL CHARGES ON THE LINKAGE OXYGEN ATOMS FOR SIX GLYCOSAMINOGLYCAN DISACCHARIDE UNITS[a]

Molecule	*Net molecular charge*	*Linkage oxygen atomic charge*
Hyaluronic acid	−1.0000	−0.2315
Chondroitin	−1.0000	−0.2238
Chondroitin 4-sulfate	−2.0000	−0.2873
Chondroitin 6-sulfate	−2.0000	−0.2777
Dermatan sulfate	−2.0000	−0.2859
Keratan sulfate	−1.0000	−0.2412

[a]Charges are given in electronic units. The net molecular charge, as indicated, is accurate to four decimal places for each glycosaminoglycan using the decomposition technique described.

distributions of α- and β-D-glucose with experimental dipole moments showed very poor agreement. However, no apparent attempt was made to consider the role of solvent in the theoretical dipole-moment calculations. There is a need for experimental dipole-moment studies on **1** in order to establish further the basis for the conformational analyses.

Calculation of the torsional potential about the linkage oxygen atoms. — Empirical potential-energy calculations do not intrinsically take into account torsional-energy contributions resulting from orbital deformation caused by bond rotation. This energy contribution can be significant for torsional rotations about a C–O bond

because the oxygen atom has a lone pair of electrons. This factor has been demonstrated for amides[40]. For disaccharides, the backbone conformational properties are principally due to torsional rotations (θ_1 and θ_2) of the $_x\mathfrak{C}^{\circ}\mathfrak{C}_y$ bridge between the monosaccharide residues. Thus, an evaluation of the torsional energy as a function of θ_1 and θ_2 may be very important in the distribution of conformational states in disaccharides. Two different torsional potential-functions have been applied to disaccharides. One is based upon the rotation barrier of dimethyl ether[17], and the other adopts the rotation barrier of methanol[27]. However, we consider that these functions are inadequate for defining the energy contribution for rotations about θ_1 and θ_2 when X and Y are different monosaccharide residues. A two-dimensional, torsional potential-function is needed. To estimate this torsional-energy contribution, conformational analyses of a disaccharide with respect to θ_1 and θ_2 can be performed as follows: First, CNDO energy contributions are used in the conformational analysis and then empirical potential energies are used. In both sets of calculations, the global minimum in energy is assigned a value of zero. The difference between the two types of energies for common values of θ_1 and θ_2 are assumed to correspond to the intrinsic torsional-energy for that particular (θ_1, θ_2) conformation. Thus, a two-dimensional, empirical, torsional potential-function can be constructed to describe the torsional energetics for the X–O–Y linkage in a disaccharide.

Torsional energy about the X–O–Y linkage in a disaccharide residue. — As previously indicated, it is not computationally practical to perform a CNDO calculation on an entire disaccharide. Furthermore, the energy contributions to the torsional energy for a rotation in the X–O–Y linkage are not significantly influenced by the entire disaccharide. The structural unit used to determine the torsional potential for the X–O–Y linkage, selected in a similar manner as for charge calculations, is shown in Fig. 5.

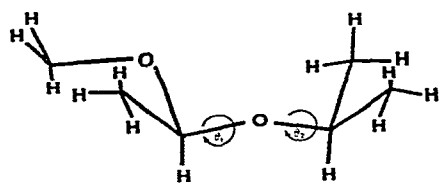


Fig. 5. Structural unit used to determine the torsional potential.

A complete conformational analysis, using empirical energy-functions, was conducted for this molecule; it included steric, coulombic, and hydrogen-bonding terms, for values of θ_1 and θ_2 from 0 to 360° in 30° intervals. The conformational energy-map is shown in Fig. 6. The CNDO procedure was then used to compute the energy of the molecule shown in Fig. 5 for those conformations within 5 kcal.mole^{-1} of the global energy-minimum found by using the empirical energy-functions. Fig. 6 also presents the difference between the energies of the empirical and CNDO

calculations on the θ_1 *vs.* θ_2 energy-surface. These represent the intrinsic torsion-energies associated with each conformation. It is worth noting that the torsional potential is not symmetric with respect to θ_1 and θ_2 and, therefore, the torsional energetics of rotation about each of the two C–O bonds are not equal. The maximum barrier-height is 7.14 kcal.mole^{-1}, or, very roughly, about 3.6 kcal.mole^{-1} for rotation about each C–O bond. As mentioned earlier, Cleland[17] adopted a symmetric, three-fold torsional potential with a barrier height of 2.4 kcal.mole^{-1} for rotation

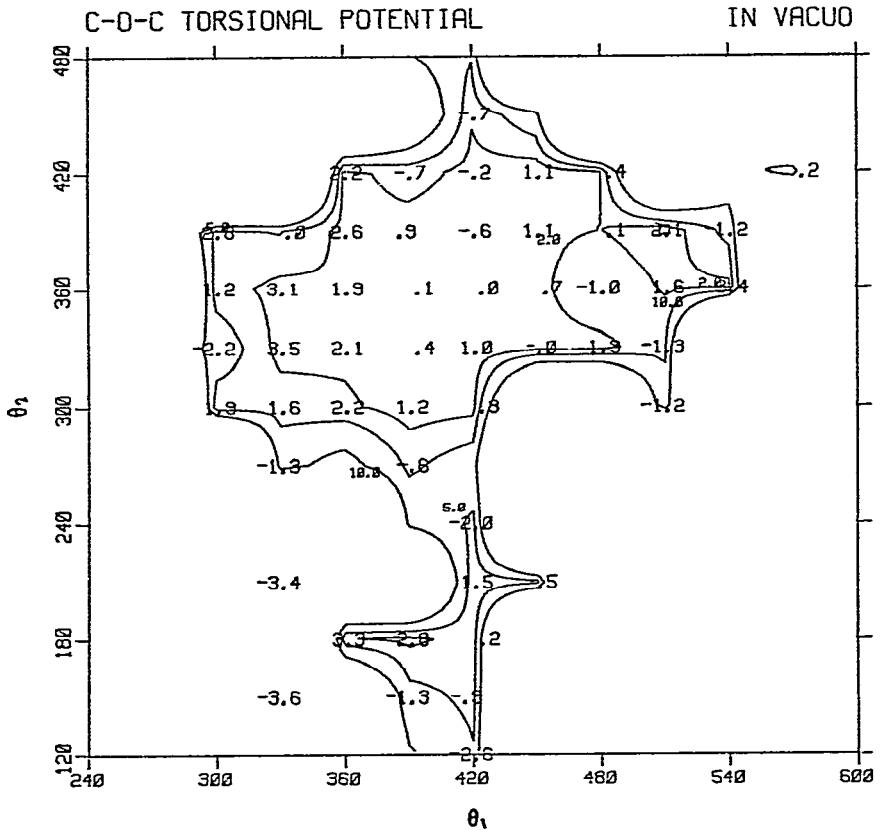
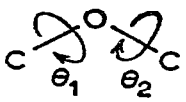
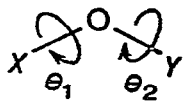


Fig. 6. Classical conformational energy-map of the molecule in Fig. 5 for rotations about the



bonds at 30° resolution. Energy minima occurred at $\theta_1 = 150°$ (510°), $\theta_2 = 30°$ (390°). Contours are plotted at 2, 5, and 10 kcal.mole^{-1} from the global minimum, as indicated. Energy contributions as a function of θ_1 and θ_2 arising from torsional energy-barriers, as described in the text, for an



linkage in kcal.mole^{-1} are superimposed at the corresponding θ_1, θ_2 location.

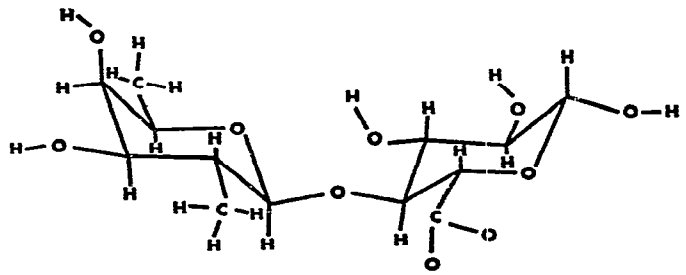


Fig. 7. Molecule used for calculation of a general steric map for a (1→4)-linked disaccharide, to serve as a steric model for the repeating units of glycosaminoglycans.

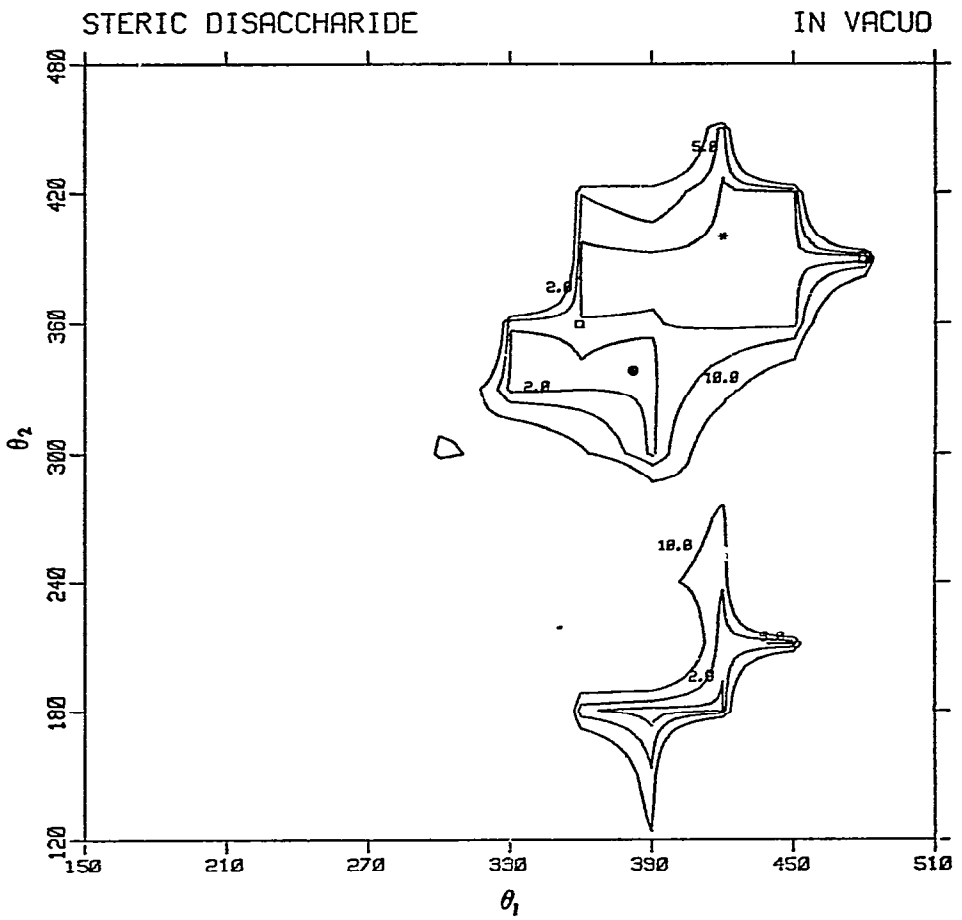


Fig. 8. Steric energy-map for the molecule in Fig. 7 using only a steric non-bonded (Lennard-Jones) potential-energy function[35] at 30° resolution. Contours plotted are 2, 5, and 10 kcal.mole^{-1} as indicated. Minima occurred at $\theta_1 = 120°$ (480°) and $\theta_2 = 30°$ (390°). Superimposed at the corresponding θ_1, θ_2 values are the predicted crystal structures of cellulose[41] (●), and xylan[42–44] (✱), and the fully extended, Meyer and Misch conformation[45] (□).

about each C–O bond. The torsional potential used[17] was determined from microwave-spectral data on dimethyl ether, a tri-symmetric molecule having only hydrogen atoms attached to the carbon atoms of the C–O–C linkage. The total barrier-height for this two-dimensional system is 4.8 kcal.mole^{-1}. This is approximately 2.35 kcal.mole^{-1} less than the maximum barrier-height calculated here. This higher barrier-potential may be rationalized as arising from the particular asymmetric substituents on the carbon atoms of the C–O–C linkage, especially the oxygen atom, which is equivalent to the ring-oxygen atom of the carbohydrate. The interaction between the lone pairs of electrons on this oxygen atom and the oxygen atom of the linkage produces a net repulsion that is reflected by the higher barrier-potential, as compared to dimethyl ether.

In order to determine the torsional energy for any (θ_1, θ_2) values, it is necessary to fit a two-dimensional empirical function to the data in Fig. 6. Attempts to fit a two-dimensional, least-square curve, by using polynomials to describe the torsional energetics, was unsuccessful. As a result, it is now suggested that linear interpolation be used to determine the torsional energy for any (θ_1, θ_2) values. We expect to describe in a later report the asymmetric, torsional potential in terms of a two-dimensional Fourier expansion.

Limiting the region of investigation for disaccharide calculations from a general steric-map. — As evident from Fig. 6, large regions of the (θ_1, θ_2) energy map are sterically disallowed. It would be highly desirable if these areas could be located for a general disaccharide of a particular linkage, regardless of the side-chains on the ring. These disallowed areas could then be disregarded in future conformational calculations. For this purpose, the molecule shown in Fig. 7 has been employed for (1→4)-linked disaccharides. A complete (θ_1, θ_2) map was constructed by using only steric, non-bonded, energy contributions. The map is shown in Fig. 8. In future conformational analyses of **1** we shall consider only those regions of the (θ_1, θ_2) map that lie within 5 kcal.mole^{-1} of the global energy-minimum for the disaccharide. Superimposed on Fig. 8 are the locations of the conformations of several β-D-(1→4)-linked polysaccharides, as suggested from X-ray analyses. It is very satisfying to observe that the experimentally suggested conformations all fall into potential-energy wells near energy minima.

Conclusions. — The results of this work lay the basis for conducting complete, solvent-dependent, conformational studies of **1**. In obtaining essential conformational data, namely charge distributions and intrinsic torsional potentials, we have presented techniques that are sufficiently general as to be applicable to other molecular species. Also, we consider that complexity of conformational analysis for **1** can be significantly reduced by constructing and using a general disaccharide map for the (1→4)-linkage. Whenever possible, the theoretical findings have been compared with experimental data. Although few experimental values are available, those found show very good agreement with theory, and offer reassurance in pursuing studies by this approach.

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