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Binding Properties of Carbohydrate *O*-Sulfate Esters Based on *ab Initio* 6-31+G** Calculations on Methyl and Ethyl Sulfate Anions

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Abstract: *Ab initio* 6-31+G** calculations on methyl and ethyl sulfate anions and four different water complexes with methyl sulfate are presented. Topological analysis of these structures demonstrates that all the terminal S═O bonds are highly polarized covalent double bonds, while the CO–S bond is a single covalent bond. As well, the cyclic nature of three bifurcated structures suggest hydrogen bonding motifs for protein–carbohydrate complexes. Systematic variations of bond angles, bond lengths, and torsion angles allowed for the parameterization of the MM2 force field. It was necessary to introduce the four atom types: sulfate (S), two terminal sulfate oxygens (O_a and O_g), and the oxygen attached to both carbon and sulfur (O_s). With these parameters satisfactory agreement between calculated and experimental values for a series of solid-state structures was found. Analysis of these calculations suggests two distinct modes of binding for *O*-sulfate groups namely, a nonspecific type that does not involve "freezing" out of the O–S bond rotations and specific hydrogen bonded structures that do "freeze" out the motions. Finally some probable conformations of a O-sulfated tetrasaccharide NOD factor for *Rhizobium meliloti* are presented.

Introduction

Most biological activities of carbohydrates depend on their ability to bind to specific receptors. Many of these carbohydrates contain negatively charged *O*-sulfate esters.[1] Several of these *O*-sulfate esters have been demonstrated to be essential for binding. The best studied case is a pentasaccharide fragment of the biopolymer heparin that binds to antithrombin III.[2]

Other examples include the following (1) heparan sulfate derived oligosaccharides that bind to basic fibroblast growth factor;[3] (2) specifically 2-O-sulfated glucuronic acid residues in heparin which bind to the nucleus of hepatoma cells;[4] (3) a dermatan sulfate derived hexasaccharide that binds to heparin cofactor II;[5] (4) sulfated Le^a and Le^x antigens on a glycoprotein that bind the cell adhesion molecule E-selectin;[6] and (5) a sulfated tetrasaccharide that is known to be a NOD factor for *Rhizobium meliloti* at nanomolar concentrations.[7]

In all these examples, specific desulfation has pronounced effects on activity. However, little is known about the precise interactions of the various sulfate groups with their respective receptors.

Among the tools available to study these interactions is theoretical chemistry. There are three levels of theoretical chemistry: molecular modeling using empirical force fields, semiempirical quantum mechanics methods, and most rigorously *ab initio* SCF (self-consistent field) methods. Due to the complexity of the systems, only empirical force fields can be used for modeling carbohydrate–receptor interactions. For the successful application of force fields extensive parameterization is necessary. Several studies have been reported using modeling techniques, but not all of the necessary structural parameters have been optimized.[8-10] These parameters include the S–O and C–OS bond lengths, and O–S–O and C–O–S bond angles, and the CC–OS, HC–OS, and CO–SO torsion angles. The use of SCF calculations to parameterize force fields is a well-established technique, for example, see ref 11.

We have chosen to study the simplest *O*-sulfate esters, methyl sulfate ($CH_3OSO_3^-$) and ethyl sulfate ($CH_3CH_2OSO_3^-$), by *ab initio* SCF methods. An earlier study of methyl sulfate anion presented a partially optimized geometry using the 4-31G basis set.[12] Since these species are both anions and contain a S(VI) atom we have chosen the 6-31+G** basis set for our study. Our study is similar to that of sulfamic acid zwitterion ($NH_3{\cdot}SO_3$) by Wiberg et al.[13] At this level, we have studied the effect of varying the bond lengths, the bond angles, and the torsion angles using the Gaussian programs package. These results have been used to derive MM2 force field parameters.

Unfortunately, there is little experimental evidence available to compare the calculated parameters with the conformational preferences or the binding modes of *O*-sulfate esters. For example, even though ^{1}H NMR data for the ring hydrogens of a particular disaccharide indicated chelation by a particular *O*-sulfate group

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to a Zn(II) ion, chelation could not be conclusively proven.[14] Similarly, a specific binding site on heparin for histamine has been postulated to include *O*-sulfate groups by interpretation of NMR binding studies.[15] More recently, the structures of a series of sulfated monosaccharides has been determined by X-ray diffraction.[16] Such studies provide the first good structural data set, and we have used this data as a test set for our calculations. The counterions to the seven sulfates in this test set are only weakly coordinating alkali metal cations. As a result, although sulfate cation interactions were observed, no conclusions about binding modes could be made from this data.

Molecular modeling of carbohydrate sulfates requires simulation of the electrostatic interactions. Two previous studies have simply distributed the net anionic charge as partial charges among the sulfate oxygens.[14,17] The latter study arbitrarily "screens" the charges by minimizing their values, in order to obtain reasonable fits to experiment. Such charge leveling is undoubtedly related to solvation by water and charge compensation by electrolytes since most interactions of interest occur in water.

We have made a preliminary study of cation binding and hydration of methyl sulfate using AM1 calculations. Our calculations suggest the importance of bifurcated hydrogen bonding by water to the sulfate oxygens and prompted a more rigorous examination by *ab initio* calculations. Finally, for further theoretical insight the charge distribution of these molecules has been analyzed using Bader's theory of atoms in molecules.[18] Such analysis has allowed for an unambiguous description of the chemical bonding in these alkyl sulfate anions and their gas-phase complexes with water.

Experimental Methods

Molecular Mechanics. All calculations used the version of MM2[19] supplied with the Hyperchem (Hypercube, Waterloo, Ontario, Canada; distributed by Autodesk, Sausalito, CA, USA) PC-based (using a 486 processor) package of programs that includes parameters published up to 1990. Our new parameters are tabulated in Table I and were entered into the program by modifying the appropriate parameter files. Minimizations used either the normal Newton–Raphson method or the Fletcher–Reeves conjugate gradient method. Monosaccharide structures were made by adding substituents one at a time to a tetrahydropyran ring optimized to a chair conformation (MM2). The monosaccharide with the correct stereochemistry was then minimized using semiempirical AM1 calculations. In this way a library of monosaccharide structures has been created which are modified as necessary with substituents for further calculations. Minimization (MM2) was carried out after the addition of each substituent. In the case of oligosaccharides each disaccharide was separately minimized in several conformations and then several combinations of these disaccharides were combined and minimized (see text).

Semiempirical. All calculations used the version of AM1[20] supplied with Hyperchem. Structures were first built and minimized using molecular mechanics without the new parameterization. This procedure essentially removes all serioues nonbonded contacts and creates "normal" sp^3, sp^2, etc. bond lengths and angles. AM1 minimization used either of the Fletcher–Reeves or Polak–Ribiere conjugate gradient methods. All systems of one ion plus water or systems with two ions plus up to six waters were minimized to a gradient of 0.05 kcal $Å^{-1}$ mol^{-1}. Larger systems were minimized to a gradient of 0.08 kcal $Å^{-1}$ mol^{-1}. A final series of single point calculations was made using the configuration interaction option of the whole system, of the ion(s) at their final coordinates without the water, and of the water at their final coordinates without the ions. The difference between the sum of the isolated systems, at their complex geometries, and the whole system was taken as the binding energy.

Table I. Calculated Parameters for *O*-Sulfate Esters for Use in MM2 Calculations

bond lengths, Å	r_0	k_s, mdyn $Å^{-1}$	bond dipole, mdyn
$S–O_a$	1.439	7.729	−3.0
$S–O_g$	1.448	13.738	−3.0
$S–O_s$	1.628	3.608	−1.0
$C–O_s$	1.461	4.350	0.44
bond angles, deg	θ_0	k_θ, mdyn Å rad^{-2}	
$O_g–S–O_s$	104.45	6.133	
$O_a–S–O_s$	102.09	1.656	
$O_g–S–O_g$	113.48	2.332	
$O_g–S–O_a$	115.17	7.078	
$S–O_s–C$	116.61	0.621	
torsion angle, deg	V_1	V_2	V_3
$CO_s–SO_a$	0.002	0.002	2.745
$HC–O_sS$	−0.016	−0.016	1.378
$CC–O_sS$	5.350	−2.155	1.212

Ab Initio. All calculations were performed on either a HP-750 or an Apollo DN 10000 minicomputer using Gaussian 88,[21] Gaussian 90,[22] and Gaussian 92[23] programs. The 6-31+G** basis set was chosen for all *ab initio* studies. The 6-31+G** basis set was derived by taking a standard split-valence basis 6-31G[24] and adding d-orbitals as the polarization functions and a simple diffuse sp shell for heavy atoms and p-orbitals at polarization functions for hydrogen atoms. It was found that the diffuse functions have a significant effect on the energies and geometries of anions because of the improved description of long-range behavior of molecular orbitals and the electron lone pairs. For all systems, full gradient geometry optimizations were carried out. The initial geometries of these optimization calculations were taken from AM1 results.

The Bader type topological analysis of charge density distribution[18] is based on the gradient vector field of the electronic density $\nabla\rho(\mathrm{r})$ and on the Laplacian of electronic density $\nabla^2\rho(\mathrm{r})$. An atom in a molecule is defined as a region of real three-dimensional space bound by a zero-flux surface of the gradient vector field of $\rho(\mathrm{r})$. Critical points in $\rho(\mathbf{r})$ are classified by the three eigenvalues $\lambda_i (i = 1, 2, \text{and } 3)$ of the Hessian matrix $(H_{ij} = \partial^2\rho(\mathrm{r})/\partial x_i\partial x_j)$. In the methyl and ethyl sulfate studied here, only three types of critical points exist that can be labeled by their rank (number of nonzero eigenvalues) and signature (excess number of positive over negative eigenvalues) are (3, −1), (3, +1), and (3, −3). A (3, −3) critical point is a local maximum that occurs generally at the nuclear position. A (3, −1) critical point, at which $\rho(\mathrm{r})$ has two negative curvatures and one positive curvature actually is a bond critical point (BCP). In other words, two interacting atoms in a molecule form a bond critical point (BCP) r_b in the electron density $\nabla\rho(\mathrm{r}) = 0$, i.e., this point r_b represents a minimum in charge density along the bond and a maximum in charge density perpendicular to the bond. The pair of gradient paths, which originate at a BCP and terminate at neighboring nuclei define a

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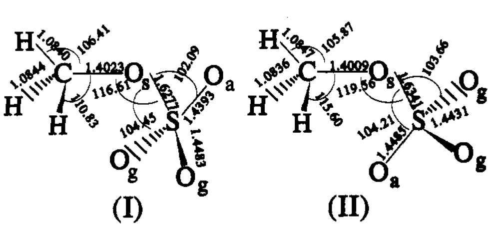


(I) (II)

Figure 1. SCF 6-31+G** staggered (I) and eclipsed (II) conformations of methyl sulfate with the important bond lengths and angles indicated.

line through the charge density distribution along which $\rho(r)$ is a maximum with respect to any lateral displacement called a bond path (in a molecule which exists in a minimum energy geometry). The necessary condition for two atoms to be bonded to one another is that their nuclei be linked by a bond path. The charge density, $\rho(r_b)$, at the bond critical point is related to the bond strength and bond order, and it is found to be significantly larger for a double bond than for a corresponding single bond. The second derivatives of ρ at the bond critical point (λ_1, λ_2, and λ_3) indicate how rapidly ρ changes on moving away from the BCP and represents the curvatures of charge density along different directions. For a normal single bond, such as the C–C bond in ethane, the two negative curvatures (λ_1 and λ_2), which are perpendicular to the bond line, are equal. However, if there is a double bond, one curvature (in the direction of the π-bond) will be much smaller than the other. This difference may be described by the elipticity of the bond, which is defined as $\epsilon = \lambda_1/\lambda_2 - 1$. For symmetrical triple bonds, since $\lambda_1 \approx \lambda_2$, ϵ is equal or close to zero. Principally, the values of $\nabla^2\rho(r)$ at the BCP are negative for a covalent interaction, and the condition $\nabla^2\rho(r) > 0$ indicates an interaction between closed-shell systems (including ionic interactions). For further differentiation, one can determine the sign of the local energy density ($H_b = G_b + V_b$, i.e., the sum of the kinetic and potential energy densities) that determines whether a bond is covalent ($H(r_b) < 0$) or ionic ($H(r_b) > 0$).[25] A (3, +1) critical point, at which $\rho(r)$ has one negative curvature and two positive curvatures, is a ring critical point. The existence of a ring critical point indicates that the molecule contains a ring structure. Bader's theory of atoms in molecules provides an umabiguous description of the molecular structure. In the present paper charge densities were obtained from the HF/6-31+G** calculations.

Results and Discussion

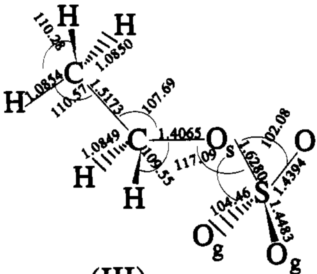
Structural Features. Figure 1 shows the SCF 6-31+G** staggered (I) and eclipsed (II) conformations of methyl sulfate along with the importance bond lengths and angles. The staggered conformation (I) is the most stable conformation at the 6-31+G** level with SCF energies of −736.575 572 2 au for (I) and −736.571 204 2 au for (II). Conformation (I) was confirmed to be a true minimum by determination of the second derivatives of the total energy. Furthermore, at this level of theory harmonic vibrational frequencies were calculated and are reported in Table II. The zero point vibrational energy (ZPVE) for I is 37.60 kcal mol^{-1}. Figure 2 shows the SCF 6-31+G** staggered conformation (III) of the homologue ethyl sulfate (−775.618 851 9 au) and Table II also gives the calculated harmonic vibration frequencies (ZPVE = 56.43 kcal mol^{-1}).

Table III shows a comparison of the key structural parameters with the AM1 results. The AM1 results exhibit marked deviations from experimental values. For example, all terminal S–O bond lengths are calculated to be too short by about 0.05 Å, see Table III. Furthermore attempts to calculate the rotational barrier about the central O–S bond suggested that all structures were almost isoenergetic. The problems associated with the AM1 calculations are at least in part a result of the minimal basis set used. In fact, several groups have demonstrated that including d-orbitals in the basis sets improves both geometries and energies of sulfur containing molecules.[26] A hypothesis, that this improvement with the inclusion of d-orbitals is not a simple result of more variables and therefore a better fit but is a direct result of the better geometry of the bonding orbitals, has been made.[27] Therefore, the basis set should include d-orbitals as in the 6-31+G** set used here (see Experimental Methods).

Table II. Methyl Sulfate (I) and Ethyl Sulfate (III): Calculated Harmonic Vibrational Frequencies at the 6-31+G** Level

mode	(I) frequencies[a]	IR intensities[b]	(III) frequencies[a]	IR intensities[b]
ν1	274.1(A′)	4.88	41.7	1.01
ν2	451.4(A′)	7.03	100.0	2.56
ν3	593.8(A′)	18.52	196.1	1.75
ν4	663.0(A′)	39.97	270.1	0.46
ν5	810.9(A′)	342.58	360.6	3.20
ν6	1118.8(A′)	227.30	435.8	3.95
ν7	1208.2(A′)	131.29	444.7	0.03
ν8	1313.19(A′)	15.44	607.8	27.70
ν9	1365.8(A′)	475.67	623.7	53.22
ν10	1595.5(A′)	4.88	682.6	95.80
ν11	1644.4(A′)	5.67	843.8	288.42
ν12	3186.6(A′)	91.25	877.1	0.04
ν13	3257.0(A′)	57.74	1004.4	74.82
ν14	92.6(A″)	3.04	1117.8	252.28
ν15	167.6(A″)	2.38	1182.2	96.97
ν16	443.2(A″)	0.09	1247.8	43.44
ν17	624.3(A″)	56.31	1288.5	0.21
ν18	1285.1(A″)	7.91	1326.6	441.37
ν19	1328.0(A″)	451.92	1365.7	474.75
ν20	1614.8(A″)	0.55	1408.8	5.73
ν21	3261.3(A″)	41.51	1514.2	2.18
ν22			1556.1	23.97
ν23			1601.6	2.27
ν24			1621.8	1.23
ν25			1664.2	0.72
ν26			3165.2	53.64
ν27			3202.7	24.17
ν28			3229.2	84.15
ν29			3234.2	0.02
ν30			3261.2	84.26

[a] In cm^{-1}. [b] In KM mol^{-1}.



(III)

Figure 2. SCF 6-31+G** conformations of staggered ethyl sulfate (III) with the important bond lengths and angles indicated.

Table III also compares the calculated structural parameters at this level with the average values for seven *O*-sulfate groups in monosaccharides.[16] Examination of Table III shows remarkable agreement between the SCF results and experiment. The only large discrepancy is between the experimental C–O_s bond length which is calculated to be too short by 0.06 Å. This difference is likely due to the substitution at carbon in the carbohydrates, i.e., methyl is not a sugar.

Since one purpose of these calculations was to parameterize a force field, all these structural parameters were varied with complete optimization of all other variables. The results were

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Table III. Methyl and Ethyl Sulfate: A Comparison of Calculated SCF-6-31+G** and AM1 Geometries with the Solid-State Structures of Methyl Sulfate and the Averages from Seven Carbohydrate *O*-Sulfate Esters

	SCF 6-31+G** $MeSO_4^-$	SCF 6-31+G** $EtSO_4^-$	AM1 $MeSO_4^-$	MM2 $MeSO_4^-$	exptl carbohydrates
			Bond Lengths, Å		
$S–O_a$	1.439	1.439	1.392	1.439	1.440 ± 0.014
$S–O_g$	1.448	1.448	1.396	1.448	1.440 ± 0.014
$S–O_s$	1.628	1.628	1.730	1.628	1.589 ± 0.016
$C–O_s$	1.403	1.407	1.385	1.464[a]	1.461 ± 0.007
			Bond Angles, deg		
$O_g–S–O_s$	104.45	104.46	102.09	104.53	106.87 ± 0.83
$O_a–S–O_s$	102.09	102.08	101.50	102.05	101.74 ± 1.00
$O_a–S–O_g$	115.17	115.14[b]	116.33	115.14	113.91 ± 1.07
$O_g–S–O_g$	113.48	113.52	114.95	113.45	112.41 ± 0.86
$S–O_s–C$	116.61	117.69	119.35	121.06	118.88 ± 1.93
			Torsion Angle, deg		
$CO_s–SO_a$	180.0 0.0(2.74)[c]	180.0	180.0 0.03(0.28)[c]	180.0	−174.43 ± 10.78

[a] Calculated using the equilibrium bond length for carbohydrates; see Table I. [b] The two O_g atoms are not exactly equivalent $\Delta\theta = 0.003°$. [c] Energy difference () in kcal mol^{-1} between eclipsed and staggered rotamers.

converted to MM2 parameters (k_s, k_θ, V_1, V_2, and V_3) by least squares fitting or using the basic program of Hopfinger and Pearlstein.[28] This formulates the bond strength, the bond bending and the bond torsion potentials with the following simple formulas. In principle any appropriate function could be fitted with this data.

$$E = k_s(r - r_0)^2$$

bond length r

$$E = k_\theta(\theta - \theta_0)^2$$

bond angle θ

$$E = V_1/2(1 + \cos\omega) + V_2/2(1 - \cos 2\omega) + V_3/2(1 + \cos 3\omega)$$

bond torsion angle ω

Some interesting observations can be made from these results. One, the three terminal oxygens of the sulfate group are not equivalent. The terminal oxygen anti to the carbon with respect to the O_s–S bond has a calculated shorter $S–O_a$ bond length than the two terminal $S–O_g$ bond lengths, and more significantly the $O_s–S–O_a$ bond angle is both theoretically and experimentally smaller than the $O_s–S–O_g$ bond angle by 2.4° and 5.1°, respectively. Two, the O_s–S bond is considerably longer then the terminal S–O bonds (about 0.2 Å). Three, stretching of any of the S–O bonds, as shown in Figure 3a, is not energetically difficult. Four, by contrast angle bending, as shown in Figure 3b, is energetically more difficult. Five, the energy barriers for rotation about either the $C–O_s$ or the O_s–S bonds are not large, as shown in Figure 4 (parts a and b). These rotational barriers are small enough that the SO_3 group should be freely rotating. Complexation processes that freeze out these motions will be very different from those that do not. Nonspecific electrostatic binding is a probable example of a complexation process that will not prevent rotation. Specific binding as in the water complexes to be discussed below requires that these motions be largely restricted.

Rotation about the $CC–O_sS$ bond determines the relative orientation of the sulfate group to the sugar. This rotation was studied by examining this bond in ethyl sulfate as shown in Figure 4b. The barriers are small (0 K gas phase). However, for a sulfate group attached to a sugar molecule other interactions with the rest of the sugar molecule will change the torsion potential. Thus, the torsion potential derived from Figure 4b (see Table I) represents an intrinsic potential. The use of calculated potentials for force field parameters for substituents, like *O*-sulfates, is adequate since, as in this example, the substituent has little effect on the sugar conformation. However, great caution must be exercised in the use of such parameters. For example, the use of the parameters in Table I for dialkyl sulfates is not recommended without extensive testing since in such a case the alkyl parameters might have to be reoptimized.

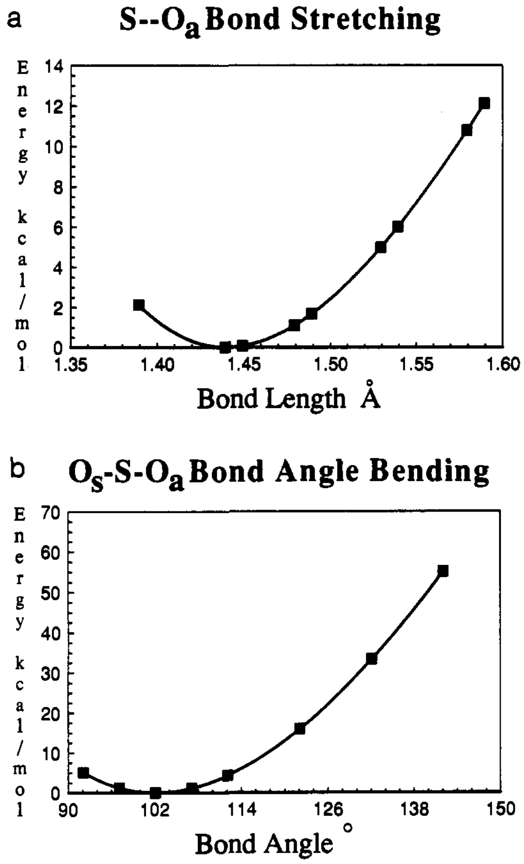

Figure 3. (a) Energy changes associated with stretching the $S–O_a$ bond and (b) the energy associated with bending the $O_a–S–O_s$ bonds. All energies form SCF 6-31+G** calculations.

Water and Counterion Binding. A preliminary study using AM1 calculations suggested that eight to nine water molecules are necessary to solvate the $CH_3OSO_3^-$ anion (ignoring solvation of the CH_3). The binding of Li^+ and Be^{2+} ions with or without water showed little difference between binding at the O_g or O_a. Experimentally, a marked preference for binding to the O_g oxygen has been reported.[29] With Be^{2+}, a chelated geometry has the lowest energy. In water, both contact ion pairs and solvent-

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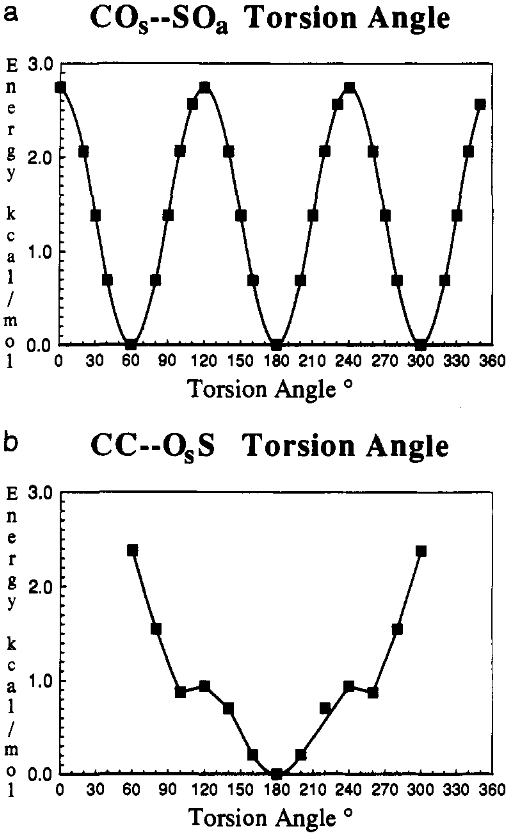

Figure 4. (a) Gas-phase (OK) torsion potentials for rotations of (a) the C–O_s–S–O_a bond and (b) the C–C–O_s–S bond. All energies form SCF 6-31+G** calculations.

separated ion pairs were found to be of comparable energy. Surprisingly, the optimum water binding geometry was a bifurcated[30] structure with two hydrogens bound to one oxygen (cf. IVa below). Typical bond lengths were 2.14 Å with an H_w–O–H_w angle of 42°. Structures with up to six bifurcated waters (two per terminal O) were found as minima. These interesting results promoted further study.

Due to computer limitations only complexes of one water and methyl sulfate (I) were studied at the *ab initio* level of theory. Four structures (IVa to IVd) were found to be true minima as confirmed by determining the Hessian eigenvalues, see Figure 5. Cyclic structure IVc with the two water hydrogens bridging the two O_g oxygens was the lowest energy, but all complexes are predicted to be stable. Even the linear hydrogen bonded complex IVb has a binding energy of 11.80 kcal mol^{-1} that is much higher than typical hydrogen bonds between neutral species and water. In the present study, it was assumed that any SCF superposition errors could be neglected for 6-31+G** calculations. Similar bifurcated water complexes with dimethyl phosphate anion have been reported.[31] These authors suggested that such well organized complexes may not form in water solution due to both their intrinsic high entropy and the necessity to break up the water structure to form them. However, these structures do suggest hydrogen bonding motifs for *O*-sulfate groups bound to proteins. In such cases the donors could be charged sites on lysine, histidine, or guanidine residues that should have even larger gas-phase binding energies. Such stabilizing interactions should be able to stop the C–O–S bond rotations and overcome the anion solvation to form stable complexes.

Topological Analysis of Bonding. The 6-31+G** bond critical point (BCP, r_b) data for Bader type topological analysis of the charge density distribution for methyl sulfate (I) and ethyl sulfate (III) are listed in Table IV. It can be seen that the $\nabla^2\rho_b$ values for I of S–O_a, S–O_g, and S–O_s are positive; however, the values of H_b, the local energy density at the BCP, are negative. Such values suggest that these S–O bonds are covalent but highly polarized. Also, the electron density at $\mathbf{r}_b$(S–O_g) and $\mathbf{r}_b$(S–O_a) has a much larger value than at r_b(S–O_s), if we compare these values with those for a typical S–O single bond in H–S–O–H (r(SO) = 1.6545 Å, ρ_b = 0.1870 au, $\nabla^2\rho_b$ = 0.2576 au, and H_b = –2.160 10 Hartree·$Å^{-3}$) and a typical S=O double bond in Me_2SO (r(SO) = 1.4906 Å, ρ_b = 0.2723 au, $\nabla^2\rho_b$ = 0.9100 au, and H_b = –2.1033 Hartree·$Å^{-3}$) calculated at the same 6-31+G** level of theory. Obviously, S–O_s is a single bond and both S–O_g and S–O_a are double S=O bonds. The same analysis shows that S–O_s of III is a single bond and S–O_g and S–O_a are double bonds. This analysis provides an unambiguous description of the bonding in *O*-sulfate ester groups.

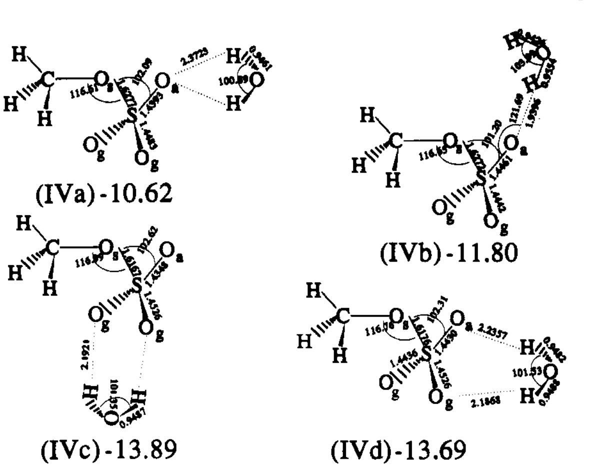

Figure 5. SCF 6-31+G** hydrogen bonded conformations of water complexes (IVa–d) with methyl sulfate(I) with the important bond lengths and angles indicated. (a) Bifurcated structure with two water protons bound to the O_a sulfate oxygen. (b) Typical linear structure with one water proton bound to the O_a sulfate oxygen. (c) Bifurcated structure with two water protons bound to the two O_g sulfate oxygens. (d) Bifurcated structure with two water protons bound to the O_a and O_g sulfate oxygens. All energies form SCF 6-31+G** calculations in kcal mol^{-1}.

Table IV. Methyl Sulfate (I) and Ethyl Sulfate (III): The Topological Properties of Charge Density Distributions at the BCPs from 6-31+G** Calculations

bond	ρ_b, au	$\nabla^2\rho_b$, au	ϵ	H_b, Hartree·$Å^{-3}$
(I) CO_s	0.2567	–0.0749	0.0110	–2.5865
(I) O_sS	0.2067	0.2563	0.0466	–1.5405
(I) SO_a	0.3034	1.4205	0.0395	–2.3358
(I) SO_g	0.2988	1.3452	0.0468	–2.3014
(I) CH_a	0.2946	–1.1319	0.0530	–2.1980
(I) CH_g	0.2978	–1.1618	0.0488	–2.2257
(III) ClO_s	0.2539	–0.0632	0.0366	–2.5439
(III) O_sS	0.2063	0.2578	0.0443	–1.5365
(III) SO_a	0.3034	1.4204	0.0394	–2.3351
(III) SO_g	0.2988	1.3455	0.0459	–2.3007
(III) $SO_{g'}$	0.2988	1.3456	0.0459	–2.3007
(III) C1C2	0.2648	–0.7350	0.0468	–1.5824
(III) C1H3	0.2867	–1.0577	0.0019	–2.1061
(III) C1H7	0.2822	–1.0210	0.0423	–2.0628
(III) C1H8	0.2867	–1.0577	0.0020	–2.1061
(III) C2H4	0.3000	–1.1729	0.0442	–2.2452
(III) C2H5	0.2999	–1.1727	0.0442	–2.2453

Based on the Bader type topological analysis of the charge density distribution, the necessary condition for two atoms to be bonded to one another is that their nuclei be linked by a bond path, i.e., a BCP exists between these two atoms. Also, the ring structure in a molecule can be confirmed by the existence of a ring critical point, i.e., a (3, +1) critical point. Four types of hydrogen-bonded complexes (IVa–IVd) between a single water

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Table V. Methyl Sulfate and Water Complexes (IVa–d): The Topological Properties of Charge Density Distributions at the BCPs and Their Hydrogen Bond Energies in kcal mol^{-1} from 6-31+G** Calculations

bond	ρ_b, au	$\nabla^2\rho_b$, au	ϵ	H_b, (Hartree·Å^{-3})
(IVa) CO_s	0.2542	–0.0516	0.0089	–2.5486
(IVa) O_sS	0.2095	0.2771	0.0499	–1.5655
(IVa) SO_a	0.3000	1.3829	0.0372	–2.2980
(Va) SO_g	0.3002	1.3674	0.0458	–2.3128
(IVa) CH_a	0.2954	–1.1393	0.0531	–2.2068
(IVa) CH_g	0.2982	–1.1660	0.0494	–2.1926
(IVa) O_wH_w	0.3857	–2.5103	0.0247	–4.7581
(IVa) O_gH_w	0.0109_1	0.0240	0.2002	0.0128
(IVa) ring	0.0109_2	0.0530		
ΔE(IVa) = –10.62 kcal mol^{-1}				
(IVb) CO_s	0.2533	–0.0419	0.0106	–2.5345
(IVb) O_sS	0.2072	0.2568	0.0466	–1.5466
(IVb) SO_a	0.2994	1.3617	0.0312	–2.2973
(IVb) SO_g	0.3010	1.3824	0.0493	–2.3196
(IVb) CH_a	0.2953	–1.1385	0.0535	–2.2061
(IVb) CH_g	0.2985	–1.1689	0.0494	–2.2355
(IVb) O_wH_w	0.3691	–2.5678	0.0234	–4.8007
(IVb) O_aH_w	0.0223	0.0762	0.0772	0.0043
ΔE(IVb) = –11.80 kcal mol^{-1}				
(IVc) CO_s	0.2525	–0.0395	0.0080	–2.5236
(IVc) O_sS	0.2117	0.2996	0.0430	–1.5824
(IVc) SO_a	0.3060	1.4616	0.0352	–2.3581
(IVc) SO_g	0.2963	1.3105	0.0452	–2.2783
(IVc) CH_a	0.2958	–1.1429	0.0534	–2.2115
(IVc) CH_g	0.2987	–1.1704	0.0498	–2.2378
(IVc) O_wH_w	0.3816	–2.5218	0.0233	–4.7662
(IVc) O_gH_w	0.0151	0.0504	0.0519	–0.0020
(IVc) ring	0.0072	0.0386		
ΔE(IVc) = –13.89 kcal mol^{-1}				
(IVd) CO_s	0.2529	–0.0428	0.0079	–2.5304
(IVd) O_sS	0.2114	0.2961	0.0499	–1.5802
(IVd) SO_a	0.3014	1.3997	0.0376	–2.3169
(IVd) SO_g[a]	0.2964	1.3101	0.0450	–2.2797
(IVd) SO_g[a]	0.3014	1.3883	0.0434	–2.3230
(IVd) CH_a	0.2958	–1.1432	0.0532	–2.2122
(IVd) CH_g	0.2984	–1.1674	0.0497	–2.2331
(IVd)CH_g[a]	0.2986	–1.1696	0.0495	–2.2372
(IVd) O_wH_w	0.3812	–2.5240	0.0233	–4.7682
(IVd) O_wH_w[a]	0.3823	–2.5107	0.0235	–4.7595
(IVd) O_gH_w	0.0151	0.0502	0.0559	–0.0020
(IVd) O_aH_w[a]	0.0138	0.0472	0.0410	–0.0003
(IVd) ring	0.0068	0.0372		
ΔE(IVd) = –13.69 kcal mol^{-1}				

[a] In complex IVd there is no mirror plane, and the two water protons are not equivalent (see Figure 5).

molecule and the methyl sulfate anion (I) were found, see Figure 5. For complex IVa, two BCPs exist between the O_a atom and each hydrogen of the water molecule forming a bifurcated hydrogen bonding system. In this complex the plane of the water molecule is perpendicular to the mirror plane of methyl sulfate (I). Inside the ring O_a–H_w–O_w–H_w of complex IVa a ring critical point of ρ(r) exists. The complex IVb is an ordinary hydrogen-bonded complex. For complexes IVc and IVd two types of bifurcated hydrogen bonds which are different from IVa were found. In these two complexes, the hydrogen atoms of water are linked either with both O_g atoms or with the O_g and O_a atoms of methyl sulfate, respectively. The cyclic structures of O_g–H_w–O_w–H_w–O_g–S or O_g–H_w–O_w–H_w–O_a–S are confirmed by the existence of ring critical points of $\rho(\mathbf{r})$. To the best of our knowledge these are the first examples of bifurcated hydrogen bonded complexes for which (3, +1) critical points have been found. The topological properties of charge distributions at the BCPs of the complexes IVa–IVd are tabulated in Table V for the values computed at the 6-31+G** level of theory. As we mentioned, the nature of these hydrogen bonds can be further investigated from the values of $\nabla^2\rho_b$ and the local energy density H_b. Generally, an ordinary hydrogen bond has a proton linked to a base atom by a closed shell interaction and to the acid atom by a shared interaction.[18b] In complexes IVa and IVb, from the calculated $\nabla^2\rho_b$ and H_b values (see Table V) the interactions between H_w and O_a are obviously closed shell interactions. On the other hand, for complexes IVc and IVd, and BCPs between protons and base atoms (O_a or O_g), the values of $\nabla^2\rho_b$ are rather small but still positive, and the values of H_b are close to zero. It seems that the diffusion of the "extra" electron (negative charge) of these complexes affects the nature of the hydrogen bond even though the closed shell interactions are maintained.

Molecular Modeling. The best test of the parameterization of a force field is comparison with known structures. We have calculated the structures of all the monosaccharides with *O*-sulfate groups with known solid-state structures. In order to generate the monosaccharides we have created a library of AM1 minimized hexose structures. Each sugar is built in its optimum chair configuration which is $^4C_1$ for most D-pyranose sugars with the correct stereochemistry at every center. This structure is first minimized using molecular mechanics and then using AM1. Such minimized structures are generally only local minima since at every ring carbon there are substituents whose orientations to the ring cannot be specified with certainty. For electronegative anomeric (C1) substituents, such as glycosides, a preference for gauche conformations is favored by the anomeric effect.[32] However, at all other centers little is known about the preferred orientations. All but one of the solid-state structures calculated here have methyl α-D-galactopyranoside as the hexose. In this case a unique hydrogen bonded conformation with OH2 bound to O1, OH3 bound to O4, and OH4 bound to O5 was found by calculation. The calculated bond lengths (std deviation 0.007 Å) and bond angles (std deviation 1.10°) compare favorably with the neutron diffraction study of this sugar.[33] The sugar crystallizes as a hydrate, and the hydrogen bonding pattern is very different so that no meaningful comparison with the calculated hydrogen bonding pattern can be made.

The three possible staggered rotamers about the exocyclic C5–C6 bond were found to be within 1 kcal mol^{-1} of each other (AM1). In other words only three minima were found for this particular sugar. Thus, the sugar skeleton for a particular sulfated sugar was selected using the C5–C6 rotamer found in the solid state and the hydroxyl rotamers as found by AM1 calculation. The favored gauche conformation for the methyl glycosides was used as this was found both experimentally and by AM1 calculation. The *O*-sulfate esters were then built onto the appropriate oxygen(s) and the structure minimized using the parameters in Table I.

Preliminary results showed that the lengthening of the C–O_s bond observed in the solid state (see Table III) was not reproduced using the optimum bond length from methyl sulfate. Thus, the equilibrium bond length was lengthened while retaining the calculated stretching constant. This change led to better values for other structural parameters of the *O*-sulfate group, perhaps by minimizing nonbonded interactions. Thus, four new atom types, *S*-sulfate, O_g-sulfate, O_a-sulfate, and O_s-sulfate, were introduced. The bond stretching, bond bending, and torsion parameters were all determined from the curve fits to the SCF methyl sulfate results except for the parameters involving O_s which do not explicitly involve the S of the sulfate. For these values the supplied MM2 parameters for an ether oxygen were used (for example the O_s–C–H bending constant). The standard deviations for bond angles, bond lengths, and torsion angles are compared with the experimental averages in Table VI. The overall agreement is good especially considering that the comparison is between calculated gas-phase and solid-state structures, in which the *O*-sulfate groups are involved in contacts with both water and counterions. The CC–O_sS torsion angle is the most affected by these interactions, and it shows the largest errors. Since this

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Table VI. A Comparison of MM2 Calculated Structures with the Solid-State Structure Averages from Seven Carbohydrate *O*-Sulfate Esters

	MM2 std dev carbohydrates	exptl mean ± std dev carbohydrates
	Bond Lengths, Å	
$S–O_a$	0.008	1.440 ± 0.014
$S–O_g$	0.010	1.440 ± 0.014
$S–O_s$	0.024	1.589 ± 0.016
$C–O_s$	0.009	1.461 ± 0.007
	Bond Angles, deg	
$O_g–S–O_s$	0.907	106.87 ± 0.83
$O_a–S–O_s$	0.615	101.74 ± 1.00
$O_a–S–O_g$	1.005	113.9 ± 1.07
$O_g–S–O_g$	0.794	112.41 ± 0.86
$S–O_s–C$	2.326	118.88 ± 1.93
	Torsion Angles, deg	
$CO_s–SO_a$	6.43	−174.43 ± 10.78
$CC–O_sS$	16.6	−154.03 ± 36.09[a]

[a] Calculated with respect to the carbon with a lower ring number than the substituent. For example an *O*-sulfate at C_2 will be defined by the torsion $C_1C_2–O_sS$.

torsion has a minimum at 180° (see Figure 4b), for *O*-sulfate groups at secondary hydroxyls two minima were observed. For example, an *O*-sulfate ester at an equatorial O-2 of a cyclic hexose could be 180° from C-1 and therefore about ±60° from C-3 or 180° from C-3 and ±60° from C-1. For unknown molecules the energy of both conformers should be determined. The $C–O_s–S$ bond angle is consistently calculated to be too large. Since this bond angle has the smallest bending force constant (see Table I) perhaps a larger value could be used.

As a test and an example of the possibilities now available we have made an investigation of some of the conformations of the sulfated tetrasaccharide GlcNR(β1,4)GlcNAc(β1,4)GlcNAc(β1,4)6SGlcNAc(α1,O)CH_3 (R is a C16 bis-unsaturated fatty acid) as a model for the NOD factor of *Rhizobium meliloti.*[7] Since there are a large number of degrees of freedom for oligosaccharides especially about the glycosidic linkages,[34] we restricted our search to only three families of structures (a, b, and c) with different glycosidic torsions about the central (third sugar to second sugar) glycosidic linkage. The initial glycosidic torsion angles correspond to three sets of minimum values found for the GlcNAc(β1,4)GlcNAc(β1,O)CH_3 disaccharide (see Experimental Methods). For each family, we considered a representative five possible orientations of the exocyclic hydroxymethylene groups for a total of 15 minimized structures. The families showed torsion angle variations for ϕ_H (H-1–C-1–O-4–C-4) and ψ_H (C-1–O-4–C-4–H-4) starting from the nonreducing termini of four to third linkage (23.8 ± 5.4° and 18.5 ± 6.2°), second to first linkage (53.2 ± 9.4° and −6.9 ± 4.7°), and the varied angles third to second linkage of (a) 23.2 ± 6.5°, 47.4 ± 3.0°, (b) 52.3 ± 3.9° and 33.1 ± 3.5°, and (c) 51.0 ± 0.5° and −158.9 ± 2.5°.

A more complete conformational analysis requires the evaluation of many more combinations of glycosidic and side-chain torsion angles. Furthermore, the fatty acid side chain likely exists in numerous interconverting conformations[19] and will confer a tendency for aggregation on the molecule. These factors were not further investigated since the prime consideration in this study is the conformation of the *O*-sulfate ester on the primary hydroxyl of the reducing end sugar. The bond lengths and bond angles for this *O*-sulfate group remained almost constant among all 15 structures and are similar to those in Table VI. The C-5–C-6–O-6–S torsion angle was remarkably constant with a mean value of −175.5 ± 3.8°. All 15 structures were within 5 kcal mol^{-1} of each other. Such a result is consistent with the existence of multiple minima conformations that freely equilibrate in the absence of external constraints, such as binding to a protein receptor. Since substitutions at the O-6 positions of either the reducing or nonreducing terminal glucosamines changes specificity[35] and by inference could be part of the protein–carbohydrate binding sites, we determined the distance between these two oxygens to be 15.4 ± 1.5 Å. Family c with the ψ_H of −158.9 ± 2.5° was at the low end of these values. Figure 6 shows the two lowest energy conformations (ΔE = 0.1 kcal mol^{-1}) found in this search, 6a from family b and 6b from family c. In the structure in Figure 6b both the *O*-sulfate and the fatty acid chain point away from the viewer, whereas in the other structure the *O*-sulfate and fatty acid are on opposite sides of the molecule. Thus, we hypothesize that protein–carbohydrate interactions, including strong hydrogen bonding to the *O*-sulfate groups, must "freeze" out the flexibility of the carbohydrate.

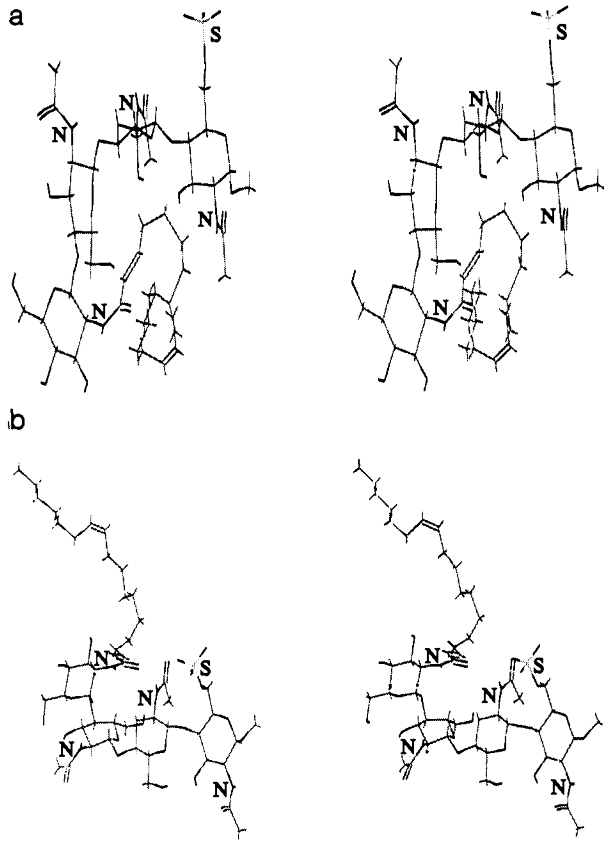

Figure 6. Two MM2 conformations of the sulfated tetrasaccharide (ΔE = 0.1 kcal mol^{-1}) GlcNR(β1,4)GlcNAc(β1,4)GlcNAc(β1,4)6SGlcNAc(α1,O)CH_3 (R is a C16 bisunsaturated fatty acid) (a) ϕ_H and ψ_H starting from the nonreducing termini (4) 19.3°, 22.8° (3) 54.5°, 34.2° (2) 63.1°, −6.9° and (b) ϕ_H and ψ_H (4) 18.4°, 23.1° (3) 50.6°, −161.6° (2) 47.9°, −5.2°.

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