Effect of Ring Distortion on the Acid Hydrolysis of 2-Methylsulfanyloxane

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Ab initio molecular orbital calculations have been used to study the effect of ring distortion on the outcome of protonation of 2-methoxyoxane and 2-methylsulfanyloxane in the gas phase. Protonation of the skew conformations of equatorial 2-methoxyoxane results in spontaneous collapse to the oxocarbenium ion, whereas the skew conformations of equatorial methyl(2-oxanyl)sulfonium exist as stable species. The chair conformation of the axial anomer of methyl(2-oxanyl)sulfonium is also found to exist as a stable species, unlike the axial anomer of methyl(2-oxanyl)oxonium, which collapses spontaneously to the oxocarbenium ion. These results are discussed in relation to the stability of thioglycosides toward cleavage by glycosyl hydrolases.

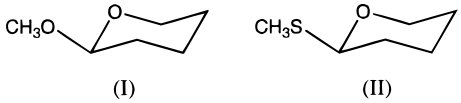
Introducion

The general mechanism of enzymatic glycoside hydrolysis, whether there is retention or inversion of anomeric configuration, takes place via two critical residues, a proton donor and a nucleophile or base.[1] Protonation of the glycosidic oxygen by the acid results in cleavage of the glycosidic bond and formation of the oxocarbenium ion. In enzymes that retain anomeric configuration, the nucleophile is believed to stabilize this ion (possibly forming a covalent intermediate), whereas in anomer-inverting enzymes, the base activates a water molecule by removing a proton. Quite often the acid and base are carboxylic acid and carboxylate groups of the enzyme respectively; however, in neuraminidase (from a variety of sources) the transition state is likely to be stabilized by a tyrosine,[2−4] the hydroxyl group of which may possibly be negatively charged.[2] In these and several other glycosyl hydrolases there does not appear any obvious candidate to play the role of the acid. In these enzymes a water molecule is believed to be the source of the proton.[5−7] There is evidence from kinetic isotope experiments that there is significant C−O bond scission in the transition state of many glycosyl hydrolases.[8−10] The extent of proton transfer to the leaving group, however, is somewhat more variable. Thus, for example, in neuraminidase from *Salmonella typhimurium*[11] and *Vibrio Cholerae*[12] there is little or no proton donation, whereas there is significant proton transfer in influenza A neuraminidase.[11] This is somewhat surprising given the high level of structural similarity in the active site of this enzyme from the three different sources. Notably, the strength of the acid is not expected to affect the degree of proton transfer; however, it is expected that the weaker the acid the longer the C−O separation.[13]

Andrews et al.[14−16] have shown that nσ^* interactions are responsible for promoting cleavage of protonated glycosides and that the equatorial anomer may undergo a conformational rearrangement that maximizes the nσ^* interaction prior to dissociation. Subsequent high-level calculations on 2-oxanol[13] have extended our understanding of the role of distortion of the pyranose ring in the mechanism of glycosyl hydrolases. For the equatorial anomer, protonation of the glycosidic oxygen of the chair conformer yields an oxonium ion in the chair conformation. Although the barrier to conversion to the oxocarbenium ion is small in the gas phase, reorganization in the enzyme active site may be difficult. Preorganization of the substrate has been shown to be a significant factor in enzymatic catalysis.[17] The $^5S_1$ and $^3S_1$ skew conformers, however, collapse directly to the oxocarbenium ion upon protonation; stable oxonium ions do not exist in the skew conformation. There is certainly no evidence for a discrete protonated species in the mechanism of glycosyl hydrolases, everything being consistent with a concerted proton transfer.[18] In addition to avoiding high-energy oxonium ion intermediates, distortion of the ring also reduces the glycosidic bond-stretch energy, thereby delaying the transition state and reducing the reaction barrier. Protonation of the axial anomer in the chair conformation leads directly to the oxocarbenium ion; there exists no stable oxonium ion in the chair conformation. Inspection of the structures of several retaining β-glycosyl hydrolases suggests ring distortion to the twist-boat conformation occurs during catalysis in these enzymes.[19]

Although the proton affinities of sulfides are greater than their ether analogues (for example, the proton affinity of dimethyl sulfide is 39.0 kJ mol^{-1} greater than that of dimethyl ether[20]), in aqueous solution sulfides are generally less basic than ethers toward proton acids (the pK_a of dimethyl sulfide and dimethyl ether is estimated to be −6.95 and −2.52, respectively[21]). It is generally accepted that the weaker basicity of thioglycosides[22] is the cause of their resistance to acid hydrolysis and consequently their ability to function as glycosidase inhibitors.[23] The slow rate of acid-catalyzed hydrolysis leading to C−S cleavage in *O,S*-acetals has been shown to be a simple consequence of the relatively low proton basicity of the sulfur.[24]

We investigate here the effect of ring distortion on the outcome of protonation of the equatorial anomers of 2-methoxyoxane (**I**) and 2-methylsufanyloxane (**II**). We also consider



(I) (II)

what happens to the axial anomer of 2-methylsufanyloxane upon protonation and how the cationic intermediates formed might influence the dissociation of the glycosidic bond.

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TABLE 1: Calculated Energies and Zero-Point Vibrational and Thermodynamic Corrections

	MP2/6-311+G(2df,p)[a]	ZPVE[b]	ΔH_{298-0}[b]	TS[b,c]	G_{298}[a]
2-oxanol					
$^1C_4$	−346.296 24	145.31	7.70	34.28	−346.177 51
$^3H_4$	−346.282 52	145.07	7.26	37.64	−346.167 83
$^3S_1$	−346.289 51	145.74	7.78	38.44	−346.174 43
$B_{1,4}$	−346.287 29	145.43	7.11	36.86	−346.171 61
$^5S_1$	−346.290 01	145.53	7.84	38.41	−346.175 05
2-oxanyl oxonium					
$^1C_4$	−346.603 43	156.67	8.42	35.52	−346.473 86
TS: $^1C_4 \rightarrow E_4$	−346.599 03	155.19	8.36	36.12	−346.471 60
E_4	−346.621 98	152.69	10.38	41.22	−346.500 13
2-methoxyoxane					
$^1C_4$	−385.494 66	172.45	9.17	41.73	−385.354 77
$^3H_4$	−385.481 22	172.13	8.74	41.36	−385.341 71
$^3S_1$	−385.489 06	172.71	9.32	42.32	−385.349 35
$B_{1,4}$	−385.486 46	172.44	8.64	40.73	−385.346 11
$^5S_1$	−385.488 97	172.59	9.34	42.25	−385.349 29
methyl(2-oxanyl)oxonium					
$^1C_4$	−385.814 79	184.42	9.77	43.13	−385.663 73
TS: $^1C_4 \rightarrow E_4$	−385.807 85	183.32	9.63	43.23	−385.658 13
E_4	−385.816 00	180.82	11.64	49.16	−385.672 70
2-methylsulfanyloxane (equatorial)					
$^1C_4$	−708.092 97	168.49	9.87	43.80	−707.958 41
$^3H_4$	−708.078 35	168.17	9.45	43.41	−707.944 14
$^0S_2$	−708.088 54	168.71	9.99	44.26	−707.954 10
$B_{1,4}$	−708.081 43	168.37	9.42	43.02	−707.946 66
$^5S_1$	−708.084 83	168.57	10.06	44.39	−707.950 59
2-methylsulfanyloxane (axial)					
$^1C_4$	−708.094 54	168.81	9.84	43.75	−708.959 64
methyl(2-oxanyl)sulfonium (equatorial)					
$^1C_4$	−708.423 33	178.45	10.20	44.37	−708.279 05
TS: $^1C_4 \rightarrow {}^0S_2$	−708.411 72	177.76	9.99	44.76	−708.268 73
$^0S_2$	−708.418 04	178.23	10.54	45.47	−708.274 74
TS: $^0S_2 \rightarrow {}^5S_1$	−708.411 78	177.35	10.22	45.01	−708.269 22
$^5S_1$	−708.416 55	178.09	10.55	45.40	−708.273 31
TS: $^0S_2 \rightarrow E_4$	−708.404 40	176.91	10.37	45.62	−708.262 74
E_4	−708.404 77	174.89	12.41	53.17	−708.270 64
methyl(2-oxanyl)sulfonium (axial)					
$^1C_4$	−708.421 90	177.83	10.59	45.53	−708.279 01
Ox^+	−270.291 83	129.50	6.68	35.57	−270.191 22
H_2O	−76.308 96	20.51	3.78	21.36	−76.305 03
CH_3OH	−115.500 60	49.41	4.29	27.00	−115.473 90
CH_3SH	−438.095 59	44.32	4.59	28.79	−438.075 48
CH_3O^-	−114.880 54	34.25	3.85	25.00	−114.867 44
CH_3S^-	−437.520 54	34.67	4.01	26.56	−437.508 42
H^+	0.000 00	0.00	2.36	12.35	−0.009 99

[a] Energies in units of hartrees. [b] Energy corrections in units of millihartrees. [c] $T = 298$ K.

Methods and Results

Standard molecular orbital calculations[25] were performed using the GAUSSIAN 94 program.[26] Calculations were performed at the MP2(fc)/6-311+G(2df,p) level upon geometries optimized at the MP2(fu)/6-31G(d) level of theory. Vibrational frequencies obtained at the HF/6-31G(d) level (after scaling by 0.8929) were used to estimate zero-point vibrational (ZPVE) and thermodynamic corrections (ΔH_{298}, S). Free energies at 298 K were evaluated as

$$G_{298} = E(\text{MP2/6-311+G(2df,p)}) + \text{ZPVE} + \Delta H_{298} - TS$$

Calculated energies and zero-point vibrational and thermodynamic corrections are provided in Table 1. Cartesian coordinates of all optimized structures at the MP2(fu)/6-31G(d) level are presented as Supporting Information. Unless otherwise noted, energies in the text refer to free energies, G_{298}, and geometries are those optimized at the MP2(fu)/6-31G(d) level.

1. Acetals as Models for Glycosides. In a series of papers by Andrews et al.[14−16] these workers were able to show that the path of proton-induced cleavage of glycosides is influenced by the conformation of the leaving group in relation to the pyranosidic oxygen. Thus, in axial glycosides the arrangement of a lone pair of electrons antiperiplanar to the glycosidic bond predisposes the acetal to cleavage to give the oxocarbenium ion (Figure 1). Conversely, it was argued that this overlap of orbitals can only be achieved by distortion of the ring of equatorial glycosides. Evidence for such stabilization was demonstrated through observation that although the geometry (at the HF/6-31G(d)level) of dimethoxymethane moved toward the oxocarbenium ion upon protonation, these changes were significantly larger for the conformation corresponding to the axial anomer.

At the MP2/6-31G(d) level, similar changes are observed, shown in Figure 1. In the pseudoaxial conformation (**III**) protonation causes a 0.08 Å decrease in the C−O_1 distance and

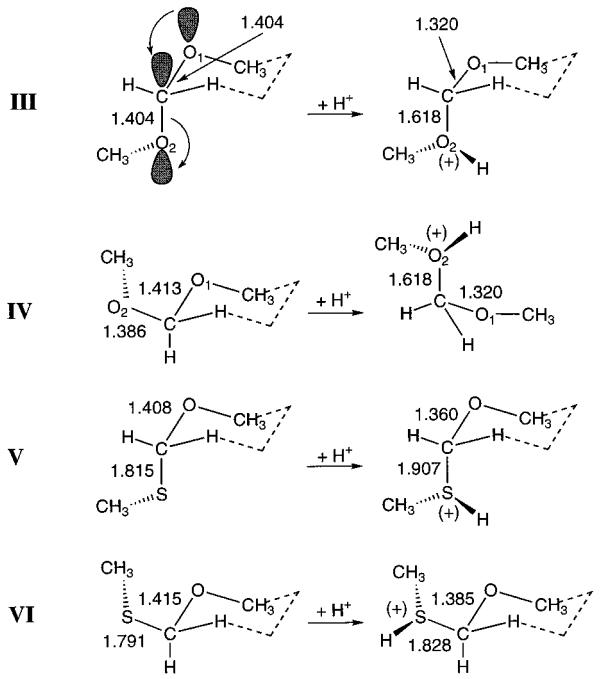


Figure 1. Optimized geometries of dimethoxymethane and methoxy(methylsulfanyl)methane (MP2(fu)/6-31G(d) level) and protonated products corresponding to axial and equatorial glycoside conformations.

a 0.22 Å increase in the C−O_2 distance. For the pseudoequatorial anomer, however, there does not exist a corresponding stable conformation (**IV**); optimization leads to the geometry obtained from the pseudoaxial anomer. Thus, at this level, a conformational change is predicted to occur spontaneously following protonation of the equatorial anomer. The results of similar calculations are also presented in Figure 1 where one of the oxygen atoms is replaced by a sulfur. Although overlap between the oxygen lone pair and the orbital on carbon might be expected to be similar to dimethoxymethane, the interaction involving sulfur is likely to be weaker. Indeed, the shortening of the C−O bond (−0.05 Å) in the pseudoaxial anomer (**V**) is slightly smaller than that observed for the C−O_1 bond in dimethoxymethane. The C−S bond, however, lengthens by less than half as much (+0.09 Å) as the C−O_2 bond in dimethoxymethane. For the pseudoequatorial conformer (**VI**) the C−O and C−S bond lengths change by only half as much again (−0.03 and 0.04 Å, respectively). Additionally, in contrast to the oxygen analogue, there does exist a stable geometry for the protonated pseudoequatorial conformer of methoxy(methylsulfanyl)methane. It would appear, therefore, that the sulfanyl analogue is not only less predisposed to cleavage but also less likely to undergo conformational change upon protonation.

2. Conformational Energies. It has been suggested, on the basis of PM3 calculations,[27] that spontaneous cleavage of glycosides may occur upon protonation of the glycosidic oxygen of equatorial glycosides, which have an antiperiplanar arrangement of the lone pair of electrons on the leaving group and the pyranosidic O−C bond, or (more importantly) through steric interference with large leaving groups. This situation would have significant implications on the mechanism of enzyme glycosidic hydrolysis. Bond cleavage could be effected without an oxonium ion intermediate in the chair conformation by simple alignment of the leaving group with respect to the pyranose ring. At the MP2/6-31G(d) level, however, the lowest energy conformation (the *stable* equatorial-chair) of the methyl(2-oxanyl)oxonium ion has an antiperiplanar arrangement of the lone pair of electrons on the leaving group and the pyranosidic C−O bond.

TABLE 2: Relative Energies (MP2/6-31G(d)) and Selected Geometric Data for the Various Rotational Conformers of Equatorial-Chair Methyl(2-oxanyl)oxonium

	ΔE^a	OCOC[b]	C−O(CH_3)[c]	O−C(OCH_3)[c]	OCO[b]
minima					
A	0.0	61.9	1.514	1.377	103.2
B	7.5	137.4	1.530	1.378	99.1
C	16.5	−38.0	1.541	1.369	101.6
D	6.1	−64.9	1.523	1.377	103.0
E	11.3	56.7	1.527	1.370	101.1
F	13.2	−158.7	1.531	1.377	98.2
transition states[d]					
AB	7.6	126.7	1.531	1.379	99.4
AC	17.3	−14.8	1.546	1.370	102.8
AE	22.2	57.7	1.502	1.375	102.7
BC	34.9	−117.9	1.539	1.368	101.2
BF	23.1	−179.5	1.511	1.377	98.6
CD	27.8	−52.1	1.517	1.372	102.1
DE	18.6	4.6	1.552	1.369	103.0
DF	14.8	−126.7	1.537	1.381	98.8
EF	25.4	124.3	1.530	1.367	101.8
TS[e]	16.4	58.7	1.614	1.343	105.9

[a] In kJ mol^{-1}. [b] In degrees. [c] In ångstroms. [d] Transition state XY connects minima **X** with **Y**. [e] Half-chair transition state from chair to oxocarbenium−methanol complex.

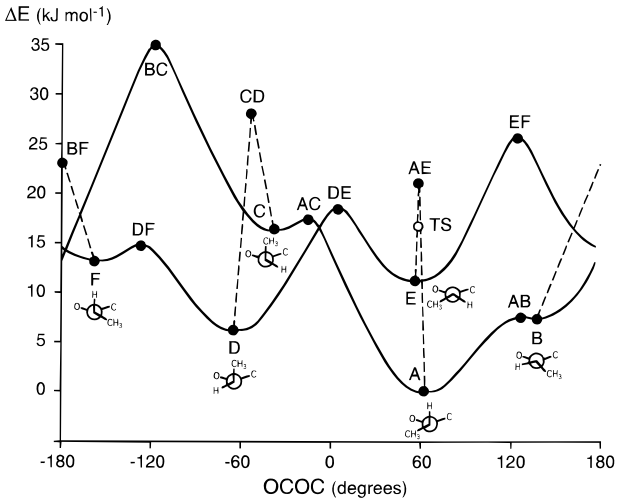
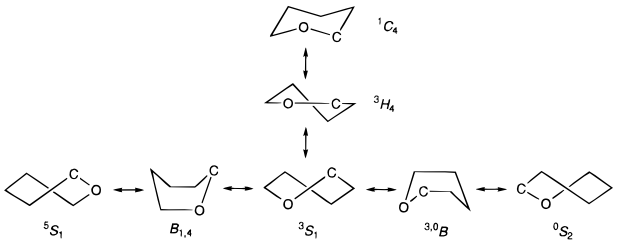


Figure 2. Schematic potential energy profile (MP2(fu)/6-31G(d) level, kJ mol^{-1}) for rotation about the glycosidic C−O bond of the chair-equatorial conformation of the methy(2-oxanyl)oxonium ion.

The lowest energy conformation of the equatorial-chair methyl(2-oxanyl)oxonium ion has both the proton and the methyl group bonded to the glycosidic oxygen gauche to the pyranosidic oxygen (**A**). There are five other minima which differ in the rotational conformation about the glycosidic C−O bond and the position of the proton (**B**−**F**). The relative energies of these six minima and the transition states interconnecting them, and selected geometrical parameters are listed in Table 2. Presented in Figure 2 is a schematic potential energy profile illustrating the relationship among these various stationary points.

The exoanomeric effect[28] describes the preference for the substituents on an anomeric center to be rotated so that the bond between the substituent and the glycosidic oxygen (R−O) and the bond between the anomeric carbon and the pyranosidic oxygen (O−C) are gauche. This arrangement aligns the O−C bond anti to a lone pair on the glycosidic oxygen and should

CHART 1



result in a shorter bond between the anomeric carbon and glycosidic oxygen and in a longer glycosidic C−O bond (when compared with geometries in which this alignment is disturbed). Structures **A** and **D** have the required geometry, are the lowest energy conformers, and have the shortest glycosidic C−O bond lengths. They do not have O−C bonds significantly longer than structures **B** and **F**, although in **C** and **E** this bond is distinctly shorter than in the other structures. The OCO angle is largest in structures **A** and **D** but smallest in structures **B** and **F**. The geometrical consequences of the exoanomeric effect have been shown to arise from the combination of many contributions.[29]

Importantly, at the MP2/6-31G(d) level spontaneous cleavage does not occur during rotation about the glycosidic bond. The glycosidic bond length varies by less than 0.04 Å between all minima and rotational transition states. The transition state for formation the oxocarbenium ion (**TS**) from **A** requires 16.4 kJ mol^{-1}, 5.8 kJ mol^{-1} lower than the energy required to invert geometry about the glycosidic oxygen between structures **A** and **E** (the OCOC dihedral angle in TS is 58.7°, similar to that found in **A** and **E**). Thus, on the basis of these calculations, the orientation of the leaving group with respect to rotation about the glycosidic bond is unlikely to play a significant role in the hydrolysis mechanism. Alignment of the leaving group may, however, have an effect on the energetics of the reaction. Tvaroska et al.[30] have indicated that large energies exist for rotation about the glycosidic bond of several glycosyl compounds.

3. Role of Ring Distortion. On the pseudorotational itinerary of pyranose rings, the chair conformation (C) can be converted to the skew (S) through the half-chair conformation (H). Interconversion between skew conformations takes place through the boat (B). Thus, the $^1C_4$ chair can be converted to the $^3S_1$ skew through the $^3H_4$ half-chair. The $^3S_1$ skew can convert to the alternate skew conformations $^0S_2$ or $^5S_1$ through the $^{3,0}B$ and $B_{1,4}$ boat conformations, respectively (see Chart 1).

3.1. Equatorial Anomers. The relative energies of the species along the path for rearrangement of the $^1C_4$ conformation to the $^5S_1$ of equatorial 2-oxanol at the level used here differ from recent G2(MP2,SVP) level results[13] by less than 2 kJ mol^{-1}. The calculated proton affinity of 2-oxanol, 781.0 kJ mol^{-1}, however, differs by almost 10 kJ mol^{-1} from that obtained at the G2(MP2,SVP) level (790.2 kJ mol^{-1}). Proton affinities at the MP2 level with large basis sets have been shown[31] to be underestimated by roughly 8 kJ mol^{-1}.

A schematic free energy profile outlining the relationship between the different conformations of 2-methoxyoxane and the cations is presented in Figure 3. The half-chair transition state in 2-methoxyoxane lies 34.3 kJ mol^{-1} above the chair, 8.9 kJ mol^{-1} higher than that found in 2-oxanol. The skew conformations lie roughly 14 kJ mol^{-1} above the chair (7 kJ mol^{-1} higher than found in 2-oxanol) and the boat approximately 8 kJ mol^{-1} above the skew conformations. Protonation of the chair conformation of 2-methoxyoxane produces the methyl-

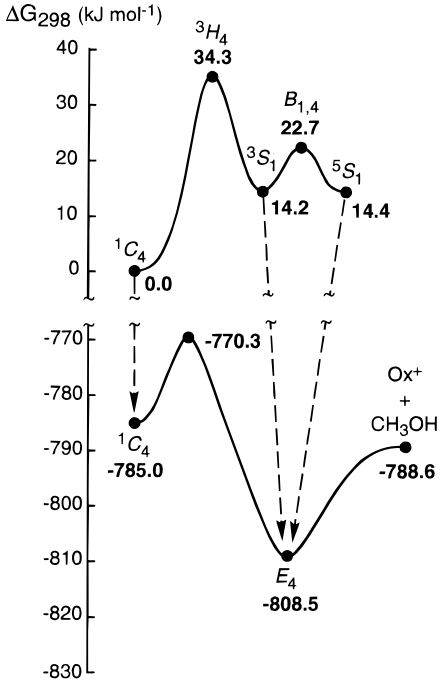
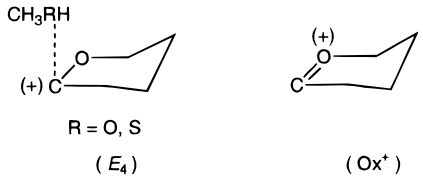


Figure 3. Schematic free energy profile (MP2/6-311+G(2df,p) level, kJ mol^{-1}, 298 K) for equatorial 2-methoxyoxane. The upper and lower curves refer to the neutral and protonated systems, respectively.

(2-oxanyl)oxonium ion in the chair conformation. The gas-phase basicity of the chair 2-methoxyoxane, 785.0 kJ mol^{-1}, is significantly greater than that of 2-oxanol, 751.9 kJ mol^{-1}. There is a 14.7 kJ mol^{-1} barrier to formation of the complex between methanol and the oxocarbenium ion (with half-boat or envelope conformation, E_4) that lies 23.5 kJ mol^{-1} lower in energy than



the chair oxonium ion. Dissociation of the complex to form the oxocarbenium ion (Ox^+) and CH_3OH requires 19.9 kJ mol^{-1}. Protonation of the two skew conformations ($^3S_1$ and $^0S_2$) leads directly to the oxocarbenium ion complex.

A schematic free energy profile for equatorial 2-methylsulfanyloxane is presented in Figure 4. The half-chair transition state lies 37.5 kJ mol^{-1} above the chair in 2-methylsulfanyloxane, very similar to the calculated barrier in 2-methoxyoxane. However, rather than leading to the $^3S_1$ skew, it connects the $^1C_4$ chair with the $^0S_2$ skew, which lies 11.3 kJ mol^{-1} above the chair. The $B_{1,4}$ boat conformation connects the $^0S_2$ skew with the $^5S_1$ skew through a barrier of 19.5 kJ mol^{-1}. Protonation of the chair conformation of 2-methylsulfanyloxane yields the methyl(2-oxanyl)sulfonium ion chair conformation. The gas-phase basicity is calculated to be 815.6 kJ mol^{-1}, 30.6 kJ mol^{-1} greater than 2-methoxyoxane. Protonation of both the $^0S_2$ and $^5S_1$ skew conformations leads to sulfonium ions in the skew conformations. The barrier to conversion of the chair to the $^0S_2$ skew is 27.1 kJ mol^{-1}. There is a barrier of 14.5 kJ mol^{-1} for conversion of the $^0S_2$ to the $^5S_1$ skew through a $B_{1,4}$ transition state. The path to formation of the complex between methylthiol and the oxocarbenium ion (E_4) from the $^0S_2$ skew has a barrier of 31.5 kJ mol^{-1}. The complex lies 22.1 kJ mol^{-1} higher in energy than the chair sulfonium ion. Dissociation to

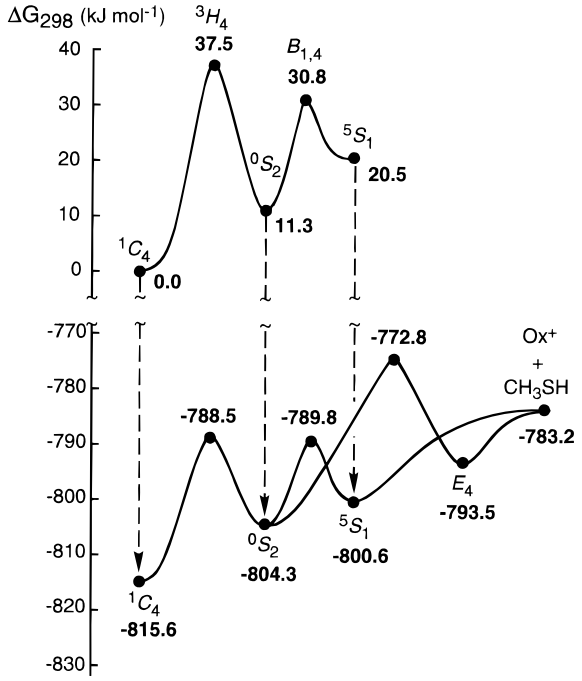


Figure 4. Schematic free energy profile (MP2/6-311+G(2df,p) level, kJ mol^{-1}, 298 K) for equatorial methyl-2-sulfanyloxane. The upper and lower curves refer to the neutral and protonated systems, respectively.

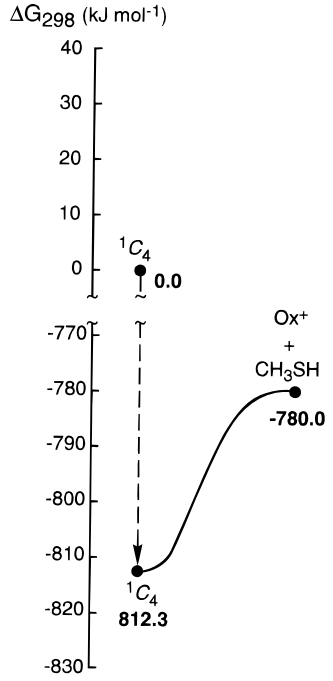


Figure 5. Schematic free energy profile (MP2/6-311+G(2df,p) level, kJ mol^{-1}, 298 K) for axial methyl-2-sulfanyloxane.

form the oxocarbenium ion and CH_3SH requires just 10.3 kJ mol^{-1}. No stable intermediate between the $^5S_1$ skew and the oxocarbenium ion could be located. Dissociation from the $^5S_1$ skew thus requires 17.4 kJ mol^{-1}.

The basicity of the skew conformations of 2-methoxyoxane and 2-methylsulfanyloxane is very similar (although the products of protonation are very different). For example, the basicities of the two $^5S_1$ conformations differ by less than 2 kJ mol^{-1} (822.9 and 821.1 kJ mol^{-1} for the methoxy- and methylsulfanyloxanes, respectively). Notably, dissociation of the $^5S_1$ conformation of methyl(2-oxanyl)sulfoniun and dissociation of the E_4 complex of methyl(2-oxanyl)oxonium differ by just 2.5 kJ mol^{-1} (17.4 and 19.9 kJ mol^{-1}, respectively).

The dissociation energy of the glycosidic bond of the chair conformations of the neutral 2-methoxyoxane and 2-methylsulfanyloxane (yielding, along with the oxocarbenium ion, the anions CH_3O^- and CH_3S^-) is 777.4 and 679.4 kJ mol^{-1}, respectively. The C−S bond is considerably weaker than the C−O bond.

For all conformations of the cationic systems, the lowest energy conformation of the leaving group was found to have the lone pair of electrons antiperiplanar to the O−C bond of the pyranosidic oxygen and anomeric carbon. The glycosidic C−O and C−S bond in the skew conformations of 2-methoxyoxane and 2-methylsulfanyloxane are roughly 0.02 Å longer than the chair. Protonation of the chair conformation of 2-methoxyoxane increases the glycosidic C−O bond by 0.12 Å, whereas in 2-methylsulfanyloxane the C−S bond lengthens by just 0.04 Å. The C−S distances in the skew conformations of the sulfonium ions are, however, roughly 0.12 Å longer than in the neutral skew conformations. In addition, the pyranosidic O−C bond in the skew conformations of the sulfonium ions is 0.03−0.05 Å longer than in the chair sulfonium ion, whereas there is no appreciable difference to be found in the different conformations of the neutral sulfanyloxanes.

3.2. Axial Anomer. The axial anomer of the chair conformation of 2-methylsulfanyloxane lies 3.2 kJ mol^{-1} lower than the equatorial anomer. A similar preference is observed for 2-methoxyoxane.[32,33] Unlike 2-methoxyoxane in which there does not exist a stable oxocarbenium ion in the chair conformation, the axial methyl(2-oxanyl)sulfonium ion does exist in the chair conformation; the sulfide analogue of 2-methoxyoxane does not spontaneously dissociate to form the oxocarbenium ion. The chair conformations of the two anomers of methyl(2-oxanyl)sulfonium are of almost identical energy. Again, no stable intermediate could be located on the path to dissociation. Dissociation from the chair to the oxocarbenium ion and CH_3SH requires 32.3 kJ mol^{-1}. In Figure 5 is presented a schematic free energy profile for the axial anomer of 2-methylsulfanyloxane.

Discussion

The gas-phase basicity and subsequent dissociation energy of the skew conformations of equatorial 2-methoxyoxane and equatorial methyl(2-oxanyl)sulfonium ions are very similar, and yet thioglycosides are not cleaved by glycosyl hydrolases. Apart from the lower basicity of the thioglycosides, it has been shown here that the glycosidic bond of thioglycosides is more stable toward dissociation following protonation than glycosides (formation of the oxocarbenium ion occurs spontaneously following protonation of the $^3S_1$ or $^5S_1$ skew conformations of equatorial 2-methoxyoxane, whereas the $^0S_2$ or $^5S_1$ skew conformations of methyl(2-oxanyl)sulfonium ion exist as stable forms). The function of enzymes that retain anomeric configuration is, at least in part, to stabilize the oxocarbenium ion intermediate.[13] For the thioglycosides, protonation does not yield the oxocarbenium ion, and therefore, the stabilization required for catalytic rate enhancement is not realized. One of the functions of anomer-inverting enzymes is to form the oxocarbenium ion to which an activated water molecule can bind. For the thioglycosides, protonation does not yield the oxocarbenium ion, and therefore, the activated water cannot bind.

The absence of an obvious candidate for the proton donor in several proteins has led to the speculation that hydrolysis might

occur via specific acid catalysis in these enzymes.[5−7] The enzyme elicits dissociation of the glycosidic bond through stabilization of the oxocarbenium ion followed by protonation of the anionic aglycon by the solvent. The transition state for acid hydrolysis of glycosides is moved from one in which C−O bond dissociation and protonation of the glycosidic oxygen occur essentially simultaneously, to one in which the C−O bond is very long and there is little proton transfer, by a substituent that decreases the proton affinity of the glycosidic oxygen. This displacement also causes an increase in the energy of the transition state. Equally, replacement of the glycosidic oxygen with sulfur, which reduces the basicity, should cause an increase in the energy of the transition state, thereby contributing to the stability of the thioglycoside.

In some enzymes the transition state includes a substantial nucleophilic component.[9] Alternatively, catalysis may also proceed through an *exploded* transition state with little or no bond formation to the nucleophile. No attempt has been made here to account of the effect of a nucleophile. Qualitatively, stabilization of the oxocarbenium ion (by the nucleophile component of an enzyme) reduces the transition-state energy and tends to decrease the extent of proton transfer in the transition state.[13]

The stability of thioglycosides (relative to glycosides) toward cleavage by acid catalysis by these enzymes therefore results from an intrinsic difference in the electronic behavior of the thioglycoside in addition to their decreased basicity. Thus, although distortion may play a critical role in the catalysis by glycosyl hydrolases, it has little effect on the thioglycosides.

Conclusions

Ab initio molecular orbital calculations at the MP2/6-311+G-(2df,p)//MP2/6-31G(d) level have been used to study the effect of ring distortion on the outcome of protonation of 2-methylsulfanyloxane. Protonation of the skew conformations of equatorial 2-methoxyoxane results in spontaneous collapse to a complex between methanol and the oxocarbenium ion, which lies 23.5 kJ mol^{-1} lower than the chair oxonium ion. In contrast, the $^{0}S_{2}$ and $^{5}S_{1}$ skew conformations of methyl(2-oxanyl)-sulfonium exist as stable species, lying 11.3 and 15.0 kJ mol^{-1} higher in energy, respectively, than the chair sulfonium ion. Dissociation from the $^{5}S_{1}$ conformation to form the oxocarbenium ion and the thiol aglycon requires 17.4 kJ mol^{-1}. There is a barrier of 31.5 kJ mol^{-1} to the formation of the oxocarbenium ion from the $^{0}S_{2}$ conformation. Protonation of the chair conformation of axial methyl(2-oxanyl)oxonium results in spontaneous cleavage, whereas the chair conformation of axial methyl(2-oxanyl)sulfonium is found to exist as a stable species. These calculations suggest that thioglycosides do not undergo acid-catalyzed cleavage by glycosyl hydrolases not simply because they are less basic than their glycosidase analogues but also because they do not undergo spontaneous cleavage upon protonation.

Supporting Information Available: Table listing calculated MP2(fu)/6-31G(d) geometries for all molecules (22 pages). Ordering information is given on any current masthead page.

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