PERKIN 2

Hydroxyoxiranone: an *ab initio* MO investigation of the structure and stability of a model for a possible α-lactone intermediate in hydrolysis of sialyl glycosides

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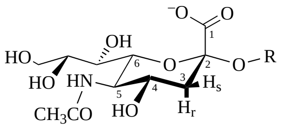
The standard enthalpy of formation $\Delta H°_{f,298}$ for hydroxyoxiranone is estimated as −377 ± 10 kJ mol^{-1} by means of *ab initio* molecular orbital calculations at the QCISD(T)(full)/6-311G(2df,p)//MP2(full)/6-311G(d,p) level of theory, corresponding to a conventional ring strain energy of 104 kJ mol^{-1}. The hydroxy substituent on C_α stabilizes the α-lactone by 65 kJ mol^{-1} with concomitant elongation of the bond from C_α to the endocyclic oxygen O_n. There is a much larger stabilization (205 kJ mol^{-1}) by the hydroxy substituent upon the zwitterion obtained by heterolysis of the C_α–O_n bond, and upon the carbene obtained by decarboxylation of the zwitterion. The relative energies of the α-lactone, zwitterion and carbene have been determined by MP2(fc)/6-31+G(d) calculations *in vacuo* and with the IPCM method for aqueous solvation. Solvation by this continuum method preferentially stabilizes the zwitterion, although at the IPCM-MP2(fc)/6-31+G(d) level the α-lactone is still 21 kJ mol^{-1} lower in energy than the zwitterion.

Introduction

Hydrolysis of glycosides of *N*-acetyl-α-D-neuraminic acid (a sialic acid) is catalysed by enzymes occurring in a wide variety of organisms, notably viral and bacterial pathogens.[1] Chong *et al.*[2] reported β-deuterium kinetic isotope effects (KIE) for hydrolysis of 4-methylumbelliferyl-*N*-acetyl-α-D-neuraminic acid (**1a**), in the pH range 6.0–9.5, catalysed by the sialidase from influenza virus, and interpreted the KIE on V_{max} at pH 6.0 measured for reaction of the [3-$^2H_2$]-substituted substrate **1a** as evidence for the intermediacy of an α-carboxylate-substituted oxocarbenium ion **2** (sialyl zwitterion). Guo *et al.*[3] determined β-deuterium KIEs for influenza-sialidase catalysed hydrolysis at pH 6.0 of a different substrate, *p*-nitrophenyl-*N*-acetyl-α-D-neuraminic acid (**1b**), containing a better leaving group. They proposed that the observed isotope effects on V_{max} and V_{max}/K_m would be consistent with a rate-limiting step which is either (i) a conformational change of the enzyme–substrate complex occurring after cleavage of the bond to the leaving group, or (ii) hydrolysis of an α-lactone intermediate **3**. Neighbouring-group participation of the α-carboxylate substituent, involving some sort of α-lactone species (like **3**), had earlier been proposed[4] for the non-enzymic hydrolysis of **1b**, and both **2** and **3** have recently been discussed[5] as possible intermediates in solvolysis of the activated sugar nucleotide **1c**.



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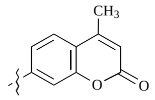

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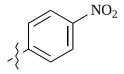

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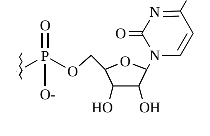

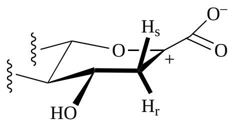

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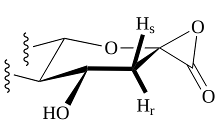

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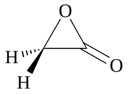
Recently in our laboratory we have performed *ab initio* MO calculations to estimate the enthalpy of formation and ring strain energy of the parent α-lactone, oxiranone **4**,[6] and to consider the mechanism for formation of this species by halide elimination from α-halocarboxylates.[7] The purpose of the present theoretical work is to investigate the stability of the unknown compound hydroxyoxiranone **5** relative to **4** and with respect to both the ring-opened zwitterion **6** and the hydroxycarbene **7** which would result from decarboxylation. These provide simple models for the species which might be produced by heterolysis of the glycon–aglycon bond in the sialyl glycosides **1a**–**c**.



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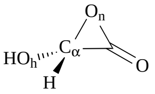

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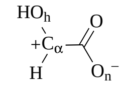

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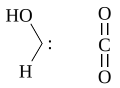

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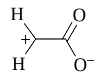

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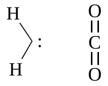

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Computational methods

Ab initio MO calculations were carried out using the Gaussian 92 and 94 series of programs[8,9] on local Silicon Graphics R4400 and Hewlett-Packard 720 workstations and on a DEC 8400 computer (Rutherford Appleton Laboratory); semi-empirical MO calculations were performed using the Mopac93 program[10] on a local Silicon Graphics R4000 workstation. Geometry optimisation for **5** was performed with the MP2(full)/6-311G(d,p) method, with a single-point energy calculation at the QCISD(T)/6-311G(2df,p)//MP2(full)/6-311G(d,p) level. Minima and saddle points were characterised by determination of their vibrational frequencies. Comparison between the energies of **5**, **6** and **7** was made at the frozen-core MP2(fc)/6-31+G(d) level, with diffuse functions on C and O to give a better description of the negative charge on the zwitterion; geometries and counterpoise corrections were determined using the HF/6-31+G(d) and MP2(fc)/6-31+G(d) methods. Single-point isodensity surface polarized continuum model (IPCM)

Table 1 Selected geometrical parameters for optimized structures, and imaginary frequencies $\nu^{\ddagger}$ for transition structures. Bond lengths in Å, angles in degrees.

Structure	Method	C_{α}–$O_{\mathbf{n}}$	C_{α}–C	C–$O_{\mathbf{n}}$	C=O	C_{α}–$O_{\mathbf{h}}$	$\angle C_{\alpha}CO_{\mathbf{n}}$/°	ΣC_{α}/°	$\angle HC_{\alpha}O_{\mathbf{h}}H$/°	$\nu^{\ddagger}$/cm^{-1}
$\mathbf{5}_{anti}$										
	HF/6-31G(d,p)	1.479	1.438	1.305	1.171	1.340	65.0	358.2	−168.8	
	HF/6-31+G(d)	1.481	1.439	1.304	1.172	1.340	65.1	358.3	−169.5	
	MP2(fc)/6-31+G(d)	1.586	1.454	1.344	1.208	1.355	68.9	359.1	−169.1	
	MP2(full)/6-311G(d,p)	1.556	1.454	1.334	1.194	1.350	67.7	359.1	−167.6	
	AM1	1.464	1.466	1.383	1.205	1.372	61.8	357.2	+165.4	
	AM1/COSMO	1.487	1.463	1.378	1.212	1.365	63.0	357.9	−179.4	
$\mathbf{5}_{gauche}$										
	HF/6-31+G(d)	1.478	1.435	1.305	1.170	1.342	65.1		+44.7	
	MP2(fc)/6-31+G(d)	1.574	1.450	1.346	1.205	1.358	68.4		+35.8	
	AM1/COSMO	1.503	1.462	1.369	1.213	1.371	64.0		+21.2	
4										
	MP2(fc)/6-31+G(d)	1.551	1.449	1.346	1.204		67.3	358.7		
$\mathbf{6}_{syn}$										
	HF/6-31+G(d)		1.598	1.213	1.203	1.250	107.4	360.0	0.0	118*i*
	MP2(fc)/6-31+G(d)		1.523	1.267	1.247	1.280	107.1	360.0	0.0	183*i*
	AM1		1.651	1.235	1.229	1.286	108.1	360.0	0.0	82*i*
	AM1/COSMO		1.522	1.264	1.262	1.286	117.1	360.0	0.0	30*i*
8										
	HF/6-31+G(d)		1.586	1.211	1.211		110.6	360.0		453*i*
	MP2(fc)/6-31+G(d)		1.445	1.281	1.281		113.1	360.0		722*i*
$\mathbf{7}_{syn}$										
	HF/6-31+G(d)					1.298			0.0	
	MP2(fc)/6-31+G(d)					1.320			0.0	
	AM1					1.280			0.0	
	AM1/COSMO					1.282			0.0	

Table 2 Calculated total energies ($E_{\mathbf{h}}$), vibrational zero-point and thermal energies ($E_{\mathbf{zp}}$ and $E_{\mathbf{th}}$, unscaled) and potential-energy change ΔE_4 for *anti* and *gauche* conformers of hydroxyoxiranone and for other species involved in isodesmic reaction (4)

	$\mathbf{5}_{anti}$	**4**	CH_4	CH_3OH	ΔE_4/kJ mol^{-1}
HF/6-31G(d,p)	−301.433 66	−226.570 24	−40.201 71	−115.046 71	−48.4
MP2(fc)/6-31+G(d)	−302.236 21	−227.190 09	−40.334 08	−115.357 84	−58.7
MP2(full)/6-311G(d,p)	−302.465 99	−227.365 21	−40.398 04	−115.474 07	−65.0
QCISD(T)(full)/6-311G(2df,p) //MP2(full)/6-311G(d,p)	−302.685 57	−227.536 96	−40.446 66	−115.570 52	−65.0
IPCM-MP2(fc)/6-31+G(d)	−302.251 33[a]				
AM1	−312.39[b]				
COSMO-AM1	−376.56[b]				
$E_{\mathbf{zp}}$/kJ mol^{-1} [HF/6-31G(d,p)]	125.59	110	124.46	114.73	
$E_{\mathbf{th}}$/kJ mol^{-1} [HF/6-31G(d,p)]	11.32	9	7.50	8.57	
	$\mathbf{5}_{gauche}$				
HF/6-31+G(d)	−301.433 59				
MP2(fc)/6-31+G(d)	−302.234 04				
IPCM-MP2(fc)/6-31+G(d)	−302.251 67[a]				
COSMO-AM1	−373.83[b]				

[a] IPCM energy at *in vacuo* geometry. [b] AM1 energy in kJ mol^{-1}.

calculations were done at the MP2(fc)/6-31+G(d)//MP2(fc)/6-31+G(d) level using a value of 78.4 for the relative permittivity of water. Semi-empirical MO calculations employed the AM1 hamiltonian and the COSMO method for aqueous solvation.

Results and discussion

Structure and stability of hydroxyoxiranone

The lowest-energy conformer of **5** has the $O_{\mathbf{h}}$–H and C_{α}–H bonds antiperiplanar, with dihedral angle $HC_{\alpha}O_{\mathbf{h}}H = -170°$ (Table 1). The only other rotamer has $HC_{\alpha}O_{\alpha}H = +36°$ and lies 5.7 kJ mol^{-1} higher in energy on the MP2/6-31+G(d) surface; although this *gauche* conformer is the better model for the spiro-fused bicyclic α-lactone species **3**, for the purpose of evaluating the enthalpy of formation for this hydroxy-substituted α-lactone the more stable *anti* conformer is considered. The standard enthalpy of formation for **5** may be calculated in the direct manner described previously for **4**.[6]

(i) The atomization energy is determined using the QCISD(T)(full)/6-311G(2df,p)//MP2(full)/6-311G(d,p) energies (Table 2) for reaction (1).

$$C_2H_2O_3 \longrightarrow 2C + 3O + 2H \qquad (1)$$

$$\Delta E_{\mathbf{atom}}(\mathbf{5}) = 2(-37.796\,42) + 3(-74.986\,01) + 2(-0.499\,81) - (-302.685\,57) = 1.135\,07\ E_{\mathbf{h}}\ (E_{\mathbf{h}} = \text{hartree})$$

(ii) Since this reaction is non-isogyric (C and O atoms each have two unpaired electron spins and atomic H has one), 12H are added to the left-hand side and are balanced on the right-hand side by $6H_2$, to yield the isogyric reaction (2). Use of the

$$C_2H_2O_3 + 12H \longrightarrow 2C + 3O + 2H + 6H_2 \qquad (2)$$

$$\text{corrected } \Delta E_{\mathbf{atom}}(\mathbf{5}) = 1.135\,07 + 6(-1.168\,32) - 12(-0.499\,81) + 6(0.174\,47)E_{\mathbf{h}} = 1.169\,67\ E_{\mathbf{h}}$$

QCISD(T)(full)/6-311G(2df,p) energies for H and H_2, together with the exact dissociation energy for H_2 (0.174 47 $E_{\mathbf{h}}$)[11] then leads to a corrected atomization energy.

(iii) The zero-point vibrational energy of $C_2H_2O_3$ [HF/6-

31G(d,p), Table 2, scaled by 0.89] is subtracted to give the dissociation energy at absolute zero (1 $E_h = 2625$ kJ mol^{-1}).

$$\Sigma D_0(\mathbf{5}) = 2959 \text{ kJ mol}^{-1}$$

(iv) Using the experimental enthalpies of formation of atoms at 0 K (C, 711; O, 247; H, 216 kJ mol^{-1}),[12] the standard enthalpy of formation for hydroxyoxiranone at 0 K is obtained from eqn. (3).

$$\Delta H^\circ_{f,0}(\mathbf{5}) = 2 \times \Delta H^\circ_{f,0}(\text{C}) + 2 \times \Delta H^\circ_{f,0}(\text{H}) + 3 \times \Delta H^\circ_{f,0}(\text{O}) - \Sigma D_0(\text{C}_2\text{H}_2\text{O}_3) \quad (3)$$
$$= 2 \times 711 + 2 \times 216 + 3 \times 247 - 2959 = -364 \text{ kJ mol}^{-1}$$

(v) Thermal corrections are added, using standard heat capacities for the elements in their standard states[13] and theoretical values from Table 2 to give: $\Delta H^\circ_{f,298}(\mathbf{5}) = -374$ kJ mol^{-1}.

Alternatively, the isodesmic eqn. (4) may be used, for which

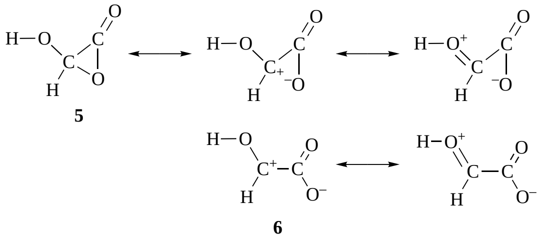
$$\mathbf{4} + \text{CH}_3\text{OH} \rightleftharpoons \mathbf{5} + \text{CH}_4 \quad (4)$$

$\Delta H^\circ_{r,298} = -62.1$ kJ mol^{-1} is calculated at the same level of theory; this value, together with literature values for the standard enthalpies of formation for gaseous methanol and methane,[12] allows $\Delta H^\circ_{f,298}(\mathbf{5})$ to be obtained as follows.

$$\Delta H^\circ_{f,298}(\mathbf{5}) = \Delta H^\circ_{r,298}[\text{eqn. (4)}] + \Delta H^\circ_{f,298}(\mathbf{4}) + \Delta H^\circ_{f,298}(\text{CH}_3\text{OH}) - \Delta H^\circ_{f,298}(\text{CH}_4) =$$
$$(-62.1) + (-190 \pm 10) + (-201.7) - (-74.5) = -379.3 \text{ kJ mol}^{-1}$$

We therefore suggest a best estimate for the standard enthalpy of formation of hydroxyoxiranone is the average of the values obtained by the two methods, *i.e.* $\Delta H^\circ_{f,298}(\mathbf{5}) = -377 \pm 10$ kJ mol^{-1}.

The energy change ΔE_4 for isodesmic eqn. (4) provides a measure of the stabilization of the oxiranone ring due to introduction of the hydroxy substituent. Electron correlation at the MP2(fc) level with the 6-31+G(d) basis increases the stabilization from -48 kJ mol^{-1} [HF/6-31+G(d)] to -59 kJ mol^{-1}; enlargement of the basis (and inclusion of core-electron correlation) further increases the stabilization to -65 kJ mol^{-1} [MP2(full)/6-311G(d,p)], but no extra stabilization is apparent from single-point calculations at the QCISD(T)(full)/6-311G(2df,p) level. These energetic stabilizations are accompanied by elongation of the $\text{C}_\alpha\text{–O}_\text{n}$ bond and by an increasing degree of planarity about C_α (as evidenced by the approach of the sum ΣC_α of the three angles $\text{CC}_\alpha\text{O}_\text{h}$, CC_αH and $\text{HC}_\alpha\text{O}_\text{h}$ towards 360°). As compared with oxiranone **4** at the MP2(fc)/6-31+G(d) level, the $\text{C}_\alpha\text{–O}_\text{n}$ bond length in **5** is also 0.035 Å longer and the deviation from planarity about C_α is smaller (Table 1). These structural changes are consistent with resonance stabilization of the hydroxyoxiranone as indicated in Scheme 1.



Scheme 1

The influence of the hydroxy substituent is to reduce the ring strain energy of the oxiranone by lengthening the endocyclic $\text{C}_\alpha\text{–O}_\text{n}$ bond distance. Since the conventional ring strain energy of oxiranone has previously been estimated[6] as 169 kJ mol^{-1}, that of hydroxyoxiranone may now be evaluated as $169 - 65 = 104$ kJ mol^{-1}, somewhat lower than the ring strain energies of oxirane (114 kJ mol^{-}) or cyclopropane (115 kJ mol^{-1}).

Structures and stability of hydroxy-substituted zwitterion

Unconstrained optimization of the hydroxyzwitterion **6** at the HF/6-31+G(d) level leads to a planar structure with the $\text{O}_\text{h}\text{–H}$ *anti* to the $\text{C}_\alpha\text{–H}$ bond and involved in an intramolecular hydrogen-bond to the carboxylate group. With the MP2/6-31+G(d) method, this species is apparently not a local minimum, for the $\text{O}_\text{h}\text{H}$ hydrogen migrates spontaneously to the carboxylate group to yield glyoxylic acid. The reverse process was studied by Bock and Redington[14] as a step in the mechanism of decarboxylation of glyoxylic acid, but only with geometries optimized at the Hartree–Fock level of theory. The planar structure **6**, in which the $\text{O}_\text{h}\text{–H}$ bond is *syn* to the $\text{C}_\alpha\text{–H}$ bond as an appropriate analogue for the cyclic zwitterion **2**, is a first-order saddle point on the potential energy surface (Table 1) and represents a transition structure for rotation about the $\text{C}_\alpha\text{–O}_\text{h}$ bond, but is stable with respect to in-plane distortions. The unsubstituted zwitterion **8** is also a first-order saddle point on the HF/6-31+G(d) and MP2(fc)/6-31+G(d) surfaces (Table 1), but is a transition structure for rotation about the $\text{C}_\alpha\text{–C}$ bond.

$$\mathbf{8} + \text{CH}_3\text{OH} \rightleftharpoons \mathbf{6} + \text{CH}_4 \quad (5)$$

The isodesmic eqn. (5) may be used with the data of Table 3 to evaluate the stabilizing influence of the hydroxy substituent upon the zwitterion, yielding $\Delta E_5 = -205$ kJ mol^{-1} at the MP2(fc)/6-31+G(d) level. The oxygen atom adjacent to the carbocationic centre of the zwitterion very effectively provides stabilization by conjugation of an electronic lone pair n_π with the vacant p_π orbital. This results in a significantly shorter $\text{C}_\alpha\text{–O}_\text{h}$ bond in the hydroxyzwitterion **6** (1.280 Å) than in the hydroxyoxiranone **5** (1.355 Å). This is consistent with resonance stabilization of the hydroxyzwitterion as indicated in Scheme 1. Bock and Redington[14] described the *anti* conformer of **6** as a weakly associated complex of hydroxycarbene and carbon dioxide with a $\text{C}_\alpha\text{–C}$ distance of 3.0 Å at the HF/6-31G(d) level. The *syn* conformer of **6** has a perfectly normal $\text{C}_\alpha\text{–C}$ bond, the length of which is reduced by electron correlation from 1.598 Å [HF/6-31+G(d)] to 1.523 Å [MP2(fc)/6-31+G(d)].

Structure and stability of hydroxycarbene

The *syn* conformer for hydroxycarbene **7** is a local minimum, lying 29 kJ mol^{-1} higher in energy than the *anti* conformer on the MP2(fc)/6-31+G(d) energy surface for the singlet state, but is the appropriate model for a glycosylidene carbene (*cf.* ref. 15). Use of isodesmic eqn. (6) with the data of Table 4 gives a value $\Delta E_4 = -191$ kJ mol^{-1} at the MP2(fc)/6-31+G(d) level for the stabilizing effect of the hydroxy substituent. The C–O bond (1.320 Å) is significantly shorter than the $\text{C}_\alpha\text{–O}_\text{h}$ bond in $\mathbf{5}_{gauche}$ (1.358 Å) as the consequence of conjugation between an oxygen lone pair and the vacant p_π orbital on carbon in the singlet carbene.

$$\mathbf{9} + \text{CH}_3\text{OH} \rightleftharpoons \mathbf{7} + \text{CH}_4 \quad (6)$$

Relative energies of α-lactone, zwitterion and carbene

At all levels of theory *in vacuo* the α-lactone, hydroxyoxiranone **5**, is the lowest energy species, despite its highly-strained three-membered ring. Electron correlation increases the stability of $\mathbf{5}_{gauche}$ relative to the hydroxyzwitterion $\mathbf{6}_{syn}$ (Table 5) from -81 (HF) to -110 kJ mol^{-1} (MP2) with the 6-31+G(d) basis using either HF or MP2 optimized geometries. The unsubstituted zwitterion **8** is even less stable (262 kJ mol^{-1}) relative to the unsubstituted α-lactone **4**. In aqueous solution it would be expected that the dipolar zwitterionic species would be

Table 3 Calculated total energies (E_h) and potential-energy change ΔE_5 for the *syn* conformer of the hydroxyzwitterion and for other species involved in isodesmic reaction (5)

Method	$\mathbf{6}_{syn}$	**8**	ΔE_5/kJ mol^{-1}
HF/6-31+G(d)	−301.402 65	−226.487 51	
HF/6-311+G(3df,2p) //HF/6-31+G(d)	−301.514 97		
MP2(fc)/6-31+G(d) //HF/6-31+G(d)	−302.184 23		
MP2(fc)/6-31+G(d)	−302.192 06	−227.090 34	−204.7
IPCM-MP2(fc)/6-31+G(d)	−302.243 50		
AM1	−251.96[a]		
COSMO-AM1	−474.82[a]		

[a] AM1 energies in kJ mol^{-1}.

Table 4 Calculated total energies (E_h) and potential-energy change ΔE_6 for the *syn* conformer of hydroxycarbene and for other species involved in isodesmic eqn. (6)

Method	$\mathbf{7}_{syn}$	**9**	CO_2	ΔE_6/kJ mol^{-1}
HF/6-31+G(d)	−113.780 69		−187.638 79	
HF/6-31+G(3df,2p) //HF/6-31+G(d)	−113.827 39		−187.709 23	
MP2(fc)/6-31+G(d) //HF/6-31+G(d)	−114.070 73		−188.112 19	
MP2(fc)/6-31+G(d)	−114.072 16	−38.975 72	−188.117 96	−190.8
IPCM-MP2(fc)/6-31+G(d)	−114.089 34		−188.121 30	
AM1	65.83[a]		−334.14[a]	
COSMO-AM1	8.99[a]		−372.00[a]	
fragments distorted as in $\mathbf{6}_{syn}$:				
HF/6-31+G(d)	−113.773 28		−187.570 60	
MP2(fc)/6-31+G(d)	−114.065 05		−188.057 49	
fragments distorted as in $\mathbf{6}_{syn}$ with ghost orbitals of counter-fragment:				
HF/6-31+G(d)	−113.775 75		−187.571 42	
MP2(fc)/6-31+G(d)	−114.073 26		−188.060 99	

[a] AM1 energies in kJ mol^{-1}.

Table 5 Relative energies of α-lactone, zwitterion and carbene + carbon dioxide; $\Delta E_{\mathbf{open}} = E(\mathbf{6}_{syn}) - E(\mathbf{5}_{gauche})$, $\Delta E_{\mathbf{diss}} = E(\mathbf{7}_{syn} + CO_2) - E(\mathbf{6}_{syn})$

Method	$\Delta E_{\mathbf{open}}$/kJ mol^{-1}	$\Delta E_{\mathbf{diss}}$/kJ mol^{-1}	BSSE corrected $\Delta E_{\mathbf{diss}}$/kJ mol^{-1}
HF/6-31+G(d)	81.2	−44.2	−52.8
HF/6-311+G(3df,2p) //HF/6-31+G(d)		−56.8	
MP2(fc)/6-31+G(d) //HF/6-31+G(d)		3.4	
MP2(fc)/6-31+G(d)	110.2	5.1	−25.7
IPCM-MP2(fc)/6-31+G(d)	21.5	84.5	
AM1	60.4[a]	−16.4	
COSMO-AM1	−101.0	111.8	

[a] $\Delta E(\mathbf{6}_{syn}) - \Delta E(\mathbf{5}_{anti})$.

stabilised. The single-point IPCM-MP2/6-31+G(d) method indeed lowers the energy of **6** relative to $\mathbf{5}_{gauche}$ by *ca.* 89 kJ mol^{-1}, although the α-lactone is still the more stable by 21 kJ mol^{-1}. (NB Geometry optimization is not implemented for this method in Gaussian 94; our attempts to re-optimize geometries using the self-consistent IPCM modification have not been successful.) The semi-empirical AM1 method predicts $\mathbf{6}_{syn}$ to be higher in energy than $\mathbf{5}_{anti}$ by 60 kJ mol^{-1} *in vacuo* (there is no energy minimum corresponding to $\mathbf{5}_{gauche}$ on the AM1 surface *in vacuo*), but using the COSMO-AM1 method for aqueous solvation, including reoptimization of the solvated species, the zwitterion $\mathbf{6}_{syn}$ is 101 kJ mol^{-1} lower in energy than the α-lactone $\mathbf{5}_{gauche}$.

To our surprise, preliminary studies on the sialyl zwitterion **2** *in vacuo* with the AM1 method (using the Spartan 3.1 program and using the molecule Builder facility)[16] indicated a spontaneous elongation of the C_α–C bond to yield a complex of sialyl carbene with carbon dioxide. Similar behaviour has been reported in a theoretical study[17] (using similar methods to those employed here) of ‘protonated pyruvate’, $CH_3C^+(O)CO_2^-$, which dissociates to a complex of methylhydroxycarbene and carbon dioxide. For the model compounds studied here, the relative energies *in vacuo* of the zwitterion and carbene + carbon dioxide vary with the level of theory used. Using either semi-empirical AM1 or *ab initio* HF/6-31+G(d) methods, the carbene + carbon dioxide combination is lower in energy than the zwitterion (by −16 and −44 kJ mol^{-1}, respectively). Electron correlation appears to stabilise the zwitterion preferentially: at the MP2/6-31+G(d) level, $\mathbf{6}_{syn}$ is lower than $\mathbf{7}_{syn}$ by 3.4 kJ mol^{-1} (HF geometries) or 5.1 kJ mol^{-1} (MP2 geometries). However, the energy difference $\Delta E_{\mathbf{diss}}$ for dissociation of the one zwitterion species into the two fragments carbene and carbon dioxide is liable to be affected by basis set superposition error (BSSE), since the energy of the adduct is artificially lowered as the result of its basis set being the sum of the basis sets for the two addends.

The BSSE for fragmentation of the hydroxyzwitterion may be estimated using a modified Boys–Bernardi counterpoise procedure which takes account of geometrical distortion.[18] First, the energies of the separate carbon dioxide and carbene fragments are determined with the geometries they adopt in the zwitterionic adduct (Table 4); the sum of these energies is $\varepsilon_{\mathbf{frag}}$. Second, the energy of each fragment is then calculated in the presence of the ghost orbitals of the counter fragment (Table 4); the sum of these energies is $E_{\mathbf{frag}}$. The BSSE is the difference $E_{\mathbf{frag}} - \varepsilon_{\mathbf{frag}}$; values of -8.6 and -30.8 kJ mol^{-1} are obtained for the HF/6-31+G(d) and MP2/6-31+G(d) methods, respectively (Table 5). The larger BSSE for the MP2 method is typical.[19] The true value of the dissociation energy of the hydroxyzwitterion with respect to the hydroxycarbene and carbon dioxide fragments, for a particular choice of basis set and theoretical method, is correctly obtained[19] by adding the BSSE to the energy difference $\Delta E_{\mathbf{diss}}$ (Table 5). At the HF/6-31+G(d) level, this gives the corrected dissociation energy as -52.8 kJ mol^{-1}, whereas at the MP2/6-31+G(d) level the effect of the counterpoise correction is to swing the result from $+5.1$ kJ mol^{-1} to -25.7 kJ mol^{-1}. An alternative to the counterpoise procedure—which corrects for the imbalance arising from use of incomplete basis sets for the fragments—is to use a more complete basis set throughout. Single-point energy calculations at the HF/6-311+G(3df,2p)//HF/6-31+G(d) level give $\Delta E_{\mathbf{diss}} = -56.8$ kJ mol^{-1}, close in value to the BSSE-corrected HF/6-31+G(d) result.

Thus, although the effect of electron correlation is to stabilize the zwitterion relative to the carbene and carbon dioxide, decarboxylative dissociation of the hydroxyzwitterion **5** to hydroxycarbene **7** is nonetheless predicted to be a favourable process *in vacuo*. The (uncorrected) MP2/6-31+G(d) value of $\Delta E_{\mathbf{diss}}$ for decarboxylation of the unsubstituted zwitterion **8** *in vacuo* is -8.8 kJ mol^{-1}.

By greatly lowering the energy of the hydroxyzwitterion, the influence of aqueous solvation is to render decarboxylation to the hydroxycarbene and carbon dioxide an unfavourable process. $\Delta E_{\mathbf{diss}}$ is 86 kJ mol^{-1} at the IPCM-MP2/6-31+G(d) level, or 112 kJ mol^{-1} according to the COSMO-AM1 method. Correction for BSSE would again reduce this energy difference by perhaps *ca.* 30 kJ mol^{-1}, but would not alter the result qualitatively.

Conclusion

The enthalpy of formation $\Delta H^\circ_{\mathbf{f,298}}$ for the more stable *anti* conformer of hydroxyoxiranone is estimated to be -377 ± 10 kJ mol^{-1}

Introduction of a hydroxy substituent at the α-carbon of oxiranone reduces the ring strain energy of the α-lactone by *ca.* 65 kJ mol^{-1}, but it stabilizes the zwitterion formed by ring-opening (heterolysis of the bond from C_α to the endocyclic oxygen) by *ca.* 205 kJ mol^{-1}. The hydroxy-substituted α-lactone is lower in energy than the zwitterion by *ca.* 110 kJ mol^{-1} *in vacuo*, and the zwitterion is also unstable with respect to the products of dissociation, a carbene and carbon dioxide, by *ca.* 26 kJ mol^{-1}. The effect of an aqueous environment is to stabilize the hydroxyzwitterion preferentially: calculations with the IPCM-MP2/6-31+G(d) method suggest that the α-lactone is still lower in energy by 21 kJ mol^{-1}, although the COSMO-AM1 method predicts the zwitterion to be the more stable in aqueous solution. Spiro-fused glycosylidene oxiranes have recently been synthesized;[20] analogous spiro-fused glycosylidene oxiranones, like structure **3**, may also be realizable.

Decarboxylation of the hydroxyzwitterion to produce hydroxycarbene is predicted to be a favourable process *in vacuo*, but not in water. Recently we have characterized a transition structure for reduction of pyruvate catalysed by lactate dehydrogenase.[21] The rate-limiting step for this enzymic reaction under normal conditions is a conformational change, preceding the chemical step, in which a loop of the protein folds over the bound substrate and cofactor, thereby excluding water from the active site. The subsequent chemical step is an acid-catalysed hydride transfer from NADH to pyruvate, which appears to involve proton transfer from the imidazolium group of a protonated histidine residue preceding hydride transfer. Why does the protonated pyruvate apparently not undergo decarboxylation in the non-aqueous environment of the active site? The answer, at least in part, may be that the carboxylate group of the pyruvate substrate (and lactate product) interacts strongly with the guanidinium group of a protonated arginine residue, and that this interaction inhibits heterolytic dissociation of the zwitterionic protonated pyruvate.

Decarboxylation of the zwitterion requires the carboxylate group to be coplanar with the incipient carbene moiety. In contrast, ring closure of the zwitterion to form the α-lactone requires the carboxylate group to be perpendicular to the plane of the trigonal α-carbon atom. These stereoelectronic requirements may dictate the course of reaction of a sialyl zwitterion **2** generated in the active site of a sialidase, and may suggest a role for the three highly-conserved arginine residues found in these enzyme active sites. Interaction between the carboxylate group of the substrate and a guanidinium group on one or other of these arginines may serve both to inhibit decarboxylation of the zwitterionic intermediate and to prevent it from attaining the necessary conformation to achieve ring closure to an α-lactone.

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