

Quantum Mechanical Analysis of an α -Carboxylate-Substituted Oxocarbenium Ion. Isotope Effects for Formation of the Sialyl Cation and the Origin of an Unusually Large Secondary ^{14}C Isotope Effect

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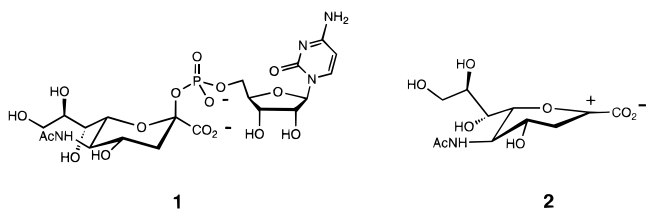
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Abstract: An *ab initio* investigation of a pyranosyl oxocarbenium ion with an α -carboxylate substituent is presented. Geometry, energetics, and isotope effects have been determined in the gas phase and with solvation models. RHF/6-31G* geometry optimizations identified a minimum for the oxocarbenium ion in which the ring atoms comprising the oxocarbenium ion lie in a plane, and the carboxylate group is twisted by 82° relative to this plane. A transition state conformer was identified which placed the carboxylate group in the oxocarbenium ion plane. The transition state conformer was ~ 5 kcal/mol higher in energy than the twist conformer (B3LYP/6-31G*/RHF/6-31G*). Single point calculations using density functional theory on the structures optimized at the RHF level indicate that in the gas phase an α -carboxylate stabilizes an oxocarbenium ion by 110 kcal/mol relative to an H-substituted oxocarbenium ion. Inclusion of solvation in the energy calculation by use of a self-consistent isodensity polarized continuum model lowers the stabilization to 17 kcal/mol in water. Equilibrium ^{14}C and ^2H isotope effects have been calculated for formation of the carboxylate-substituted oxocarbenium ion and are compared to the experimentally determined kinetic isotope effects for acid-catalyzed solvolysis of cytidine monophosphate *N*-acetylneuraminic acid (CMP-NeuAc) (Horenstein, B. A.; Bruner, M. *J. Am. Chem. Soc.* **1996**, *118*, 10371–10379). The calculations support a transition state model which features a nearly fully formed sialyl oxocarbenium ion with the carboxylate group planar or near planar to the oxocarbenium ion plane. A rationale is provided to explain the origin of an unusually large secondary ^{14}C KIE at the carboxylate carbon of CMP-NeuAc.

Introduction

Cytidine monophosphate *N*-acetylneuraminic acid¹ (CMP-NeuAc, **1**) is the donor of sialic acid (i.e., *N*-acetylneuraminic acid) for the sialyltransferase-catalyzed biosynthesis of sialylated oligosaccharides found in many glycoproteins and glycolipids of a variety of species, including mammals.^{2,3} The structure of the



NeuAc glycon portion of **1** is unusual for a monosaccharide in that it has a carboxylate group adjacent to the anomeric center. Because of the chemical novelty of **1** and its central role in biosynthesis of sialylated oligosaccharides, we are interested in the solution chemistry and enzymology of this compound. A commonly identified theme for glycohydrolases and solution hydrolysis of glycosides has been the observation that these reactions involve formation of oxocarbenium ions or transition states having oxocarbenium ion character.⁴ This is also the case for the acid-catalyzed solvolysis of CMP-NeuAc since this

reaction proceeds with a secondary β -dideuterium kinetic isotope effect between 1.20 and 1.25;⁵ kinetic isotope effects of this size may be attributed to hyperconjugation and are diagnostic for oxocarbenium ion character in the transition state.⁶ A central issue is to define how the carboxylate may be involved in the chemistry of the sialyloxocarbenium ion. Studies of α -substituent effects on carbenium ion forming reactions have been investigated,⁷ but oxocarbenium ions with α -carboxylate substitution as in the sialyl cation **2** have not yet been characterized. Oxocarbenium ions with α -carboxylate substitution have been proposed for influenza neuraminidase, KDO-8-phosphate synthase, and UDP-GlcNAc enolpyruvyl transferase,^{8–10} providing a significant impetus for the characterization of this species.

Figure 1 provides some gross features for possible pathways⁵ of the acid-catalyzed solvolysis of CMP-NeuAc, which proceeds to afford CMP and NeuAc. Both paths are initiated by

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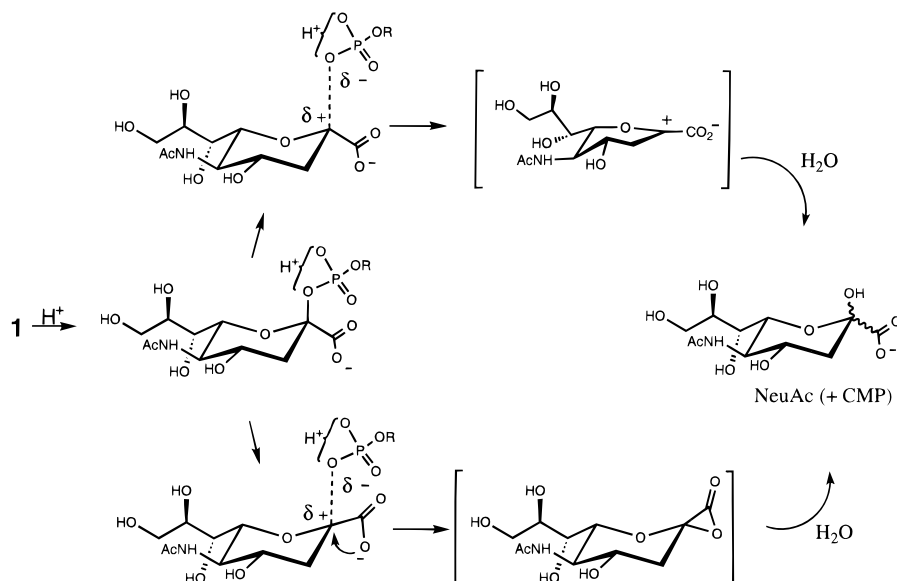


Figure 1. Two possible pathways for acid-catalyzed solvolysis of CMP-NeuAc (**1**).

protonation, presumably at the glycosidic phosphate oxygen. The upper path involves protonation at phosphate, scission of the glycosidic bond, and loss of CMP to reach a transition state having considerable oxocarbenium ion character. The transition state forms either a stable oxocarbenium ion or short-lived ion pair which is subsequently attacked by solvent to yield *N*-acetylneuraminic acid. The lower path involves nucleophilic participation of the carboxylate group to assist departure of protonated CMP. The α -lactone-like transition state collapses to the α -lactone, which is subsequently solvolyzed to the product *N*-acetylneuraminic acid. It is interesting that this scheme finds some parallel in the historical and contemporary question regarding the mechanism of retaining glycosylases, the archetype being lysozyme; there has been considerable debate regarding whether an active site carboxylate residue proximate to an oxocarbenium ion-like transition state would trap the transition state or stabilize formation of an oxocarbenium ion intermediate.^{4a,c,11}

The acid-catalyzed aqueous methanolysis of **1** proceeds to afford approximately equal amounts of α - and β -*N*-acetylneuraminic acid methyl glycosides, suggesting that this reaction forms either a solvent equilibrated oxocarbenium ion or an ion-pair intermediate. The aqueous acid-catalyzed solvolysis proceeds with a primary ¹⁴C KIE of 1.03.⁵ Combined with the large β -secondary-deuterium KIE's discussed above, the data argue for a transition state which must be close in structure to a fully formed oxocarbenium ion, with little or no nucleophilic participation of the carboxylate group. Any significant nucleophilic role for the carboxylate group should impart S_N2 character to the reaction coordinate, reducing the β -²H and raising the primary ¹⁴C KIE's.¹² An unusually large¹² secondary ¹⁴C KIE of 1.037 was observed at the carboxylate carbon,⁵ which implies that the overall vibrational environment of the carboxylate gets substantially looser in proceeding from the ground state to the transition state.

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In this work, *ab initio* calculations are presented which examine structure, bonding, energetics, and equilibrium isotope effects for formation of a pyranosyl α -carboxylate oxocarbenium ion **3**, with the carboxylate in a conformation coplanar with the oxocarbenium ion plane; a rotameric isomer **4**, with the carboxylate plane twisted with respect to the oxocarbenium ion plane; and an α -lactone **5**, which would result from back-side attack of the carboxylate at the anomeric carbon of CMP-NeuAc. These three species were chosen to represent different features of possible transition states for solvolysis of CMP-NeuAc. Results are reported for gas-phase calculations and those which model an aqueous environment. These studies suggest that an electrostatically driven geometric bond distortion is the origin of the very large ¹⁴C secondary KIE and provide a model of the transition state for the acid-catalyzed solvolysis of CMP-NeuAc.

Methods

Ab initio calculations were performed using Gaussian 94,¹³ revision C.3, on a Silicon Graphics Indigo XZ workstation and an IBM RS 6000 SP. Calculations at the RHF level employed 6-31G*, 6-31+G*, or 6-31G** basis sets. Density functional theory (DFT) calculations employed the Becke 3 parameter exchange functional¹⁴ and the Lee–Yang–Parr correlation functional;¹⁵ the 6-31G* basis set was used for this level of theory. Semiempirical calculations were performed with MOPAC¹⁶ and used MNDO parameters.¹⁷ For geometry optimizations and frequency calculations which modeled aqueous solvation, a self-consistent reaction field¹⁸ was used with a dielectric constant of 78 and a molar volume as calculated within Gaussian 94. Isotope effects

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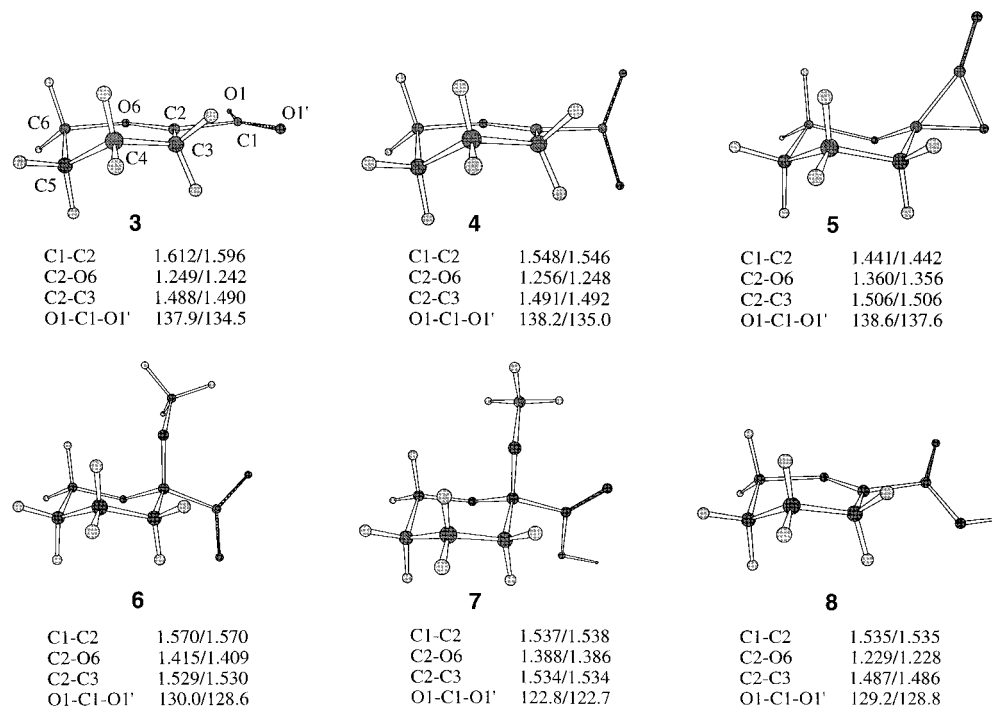


Figure 2. Structures and selected geometric parameters for compounds **3–8**. The structures presented are RHF/6-31G* minima or transition states. The atom numbering scheme for compound **3** is representative. The geometric parameters listed under each structure are in units of angstroms or degrees; the first number is the gas-phase value, and the second number is the value calculated with the SCRF aqueous solvation model. The Supporting Information contains Cartesian coordinates for these structures.

Table 1. Energies for Planar and Twisted Oxocarbenium Ions **3** and **4** and α -Lactone **5**^a

method ^{a,b}	3 (planar)	4 (twist)	5 (α -lactone)
RHF	-456.263 00 (7.0)	-456.266 15 (5.0)	-456.274 10 (0.0)
B3LYP	-458.925 06 (9.0)	-458.933 53 (3.7)	-458.939 47 (0.0)
MP2	-457.547 01 (14.2)	-457.551 79 (11.2)	-457.569 70 (0.0)
RHF/SCI-PCM ^c	-456.300 22 (0.4)	-456.300 92 (0.0)	-456.284 79 (10.1)
B3LYP/SCI-PCM	-458.951 58 (5.1)	-458.959 63 (0.0)	-458.947 25 (7.8)
MP2/SCI-PCM	-457.578 7 (0.9)	-457.580 12 (0.0)	-457.577 21 (1.8)

^a The single point calculations employed the structures for **3–5** obtained by full minimization at RHF/6-31G*. ^b Energies are in hartrees and include ZPE determined at RHF/6-31G*. The numbers in parentheses refer to the relative energies in kcal/mol of **3–5** for a given method. ^c An aqueous environment was modeled with a self-consistent isodensity polarized continuum model²⁰ and a dielectric of 78.

were calculated with QUIVER,¹⁹ which used as input the Cartesian force constants obtained from Gaussian 94 frequency calculations. Frequencies calculated with QUIVER were scaled by 0.9.

Evaluation of the relative energies of species in the gas phase or an aqueous environment was performed in the following way. Geometries were optimized to stationary points at RHF/6-31G* theory; frequencies were calculated and scaled by 0.9 for the zero-point energy (ZPE) correction. The ZPE was added to the electronic energy determined at B3LYP/6-31G* or MP2/6-31G* theory; for modeling an aqueous environment, B3LYP/6-31G* or MP2/6-31G* theory was used in conjunction with the self-consistent isodensity polarized continuum model²⁰ (SCI-PCM) provided in Gaussian 94.

Model Structures. The large number of heavy atoms in CMP-NeuAc (**1**) and the sialyloxocarbenium ion (**2**) made *ab initio* calculations on these structures computationally inaccessible, necessitating that model compounds **3–8** be used for the calculations. Compounds **3** and **4** are models for a sialyloxocarbenium ion with the carboxylate group residing in and twisted out of the oxocarbenium ion plane, respectively. Compound **5** was used to model the α -lactone that would result from back-side attack of the carboxylate group on the anomeric carbon of CMP-NeuAc. Compound **6** is a model for CMP-NeuAc and was required as the reference reactant state for calculation of equilibrium isotope effects. Compounds **7** and **8** were the carboxylate protonated analogues of compounds **6** and **3**, respectively; they were used to assess the effect of protonation on isotope effects at the carboxylate carbon. All starting structures were fully optimized to minima or transition states as noted in the text. For all basis sets and RHF or RHF/SCRF theories, reactant state **6/7** minimized

to a ¹C₄ conformation; compound **5** minimized to a flattened ¹C₄ conformation. The oxocarbenium models **3**, **4**, and **8** optimized to very similar conformers planar about the oxocarbenium ion atoms, best described as an E₄ sofa but with a slight amount of ³H₄ character. The minima for **3**, **4**, and **8** were quite similar to the results reported for optimization of (tetrahydropyranosyl)oxocarbenium ions.²¹

Results and Discussion

Geometries and Energetics of Reaction Coordinate Species. For interpretation of isotope effects it is the *change* in geometric parameters (or more accurately, force constants)²² which is important; these data are considered later. Several aspects of the geometric parameters²³ for the sialyl cation models bear mention; the results for the 6-31G* basis set are representative (Figure 2). The calculated energies for **3–5** are presented in Table 1. The “twist” oxocarbenium ion **4** minimized to a conformation having a dihedral angle of 81.7° between one of the carboxylate oxygens and the oxocarbenium ion ring oxygen.

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Table 2. Changes in Geometric Parameters and Isotope Effects for Formation of Oxocarbenium Ions or an α -Lactone^a

process	geometric parameters ^{b,c}				isotope effects ^{b,c}		
	Δr_{C2-O6} , Å	Δr_{C2-C3} , Å	Δr_{C-COO} , Å	$\Delta \theta_{OCO}$, deg	1- ¹⁴ C	2- ¹⁴ C	3, ³ - ² H
experiment					1.036 ± 0.004	1.030 ± 0.004	1.20–1.25
6 → 3	-0.166	-0.041	0.041	7.9	1.0347	1.0267	1.1705
6 → 3 ^d	-0.167	-0.040	0.026	5.8	1.0254	1.0183	1.1652
6 → 8 ^d	-0.181	-0.044	-0.035	0.2	1.0008	0.9960	1.1703
7 → 3 ^d	-0.144	-0.044	0.058	11.7	1.0312	1.0305	1.2074
7 → 8 ^d	-0.158	-0.048	-0.003	6.1	1.0065	1.0080	1.2127
6 → 4	-0.159	-0.038	-0.023	8.2	1.0199	1.0166	1.1304
6 → 4 ^d	-0.161	-0.038	-0.024	6.4	1.0133	1.0110	1.1448
6 → 5	-0.055	-0.023	-0.130	8.6	1.0229	1.0084	1.0272
6 → 5 ^d	-0.053	-0.024	-0.128	9.0	1.0236	1.0138	1.0136

^a Each line in the table represents a particular "reaction" evaluated at RHF/6-31G* theory. ^b See compound **3** in Figure 2 for the atom numbering scheme. ^c Calculated for 310 K. ^d Calculated with SCRf aqueous solvation model. The experimental data are from ref 5.

As anticipated for an oxocarbenium ion, the dihedral angle about the C2–O6 bond was near zero at 0.3°, and the C2–O6 bond length was 1.256 Å, reflecting the delocalization of O6 nonbonding electrons into the vacant p-orbital at C2. The C2–C1 bond between the positively charged former anomeric carbon and the carboxylate is 1.548 Å. Associated with this is a wide OCO carboxylate angle of 138.2°. The geometric parameters for the twisted cation were relatively insensitive to the SCRf solvation model; however, the carboxylate OCO bond angle was smaller than the gas phase value (135.0° versus 138.2), possibly reflecting a reduction of electron repulsion between the two carboxylate oxygen atoms.

The planar oxocarbenium ion **3** is not a local minimum on the potential energy surface for any basis set employed, but rather a transition state corresponding to rotation about the C–COO⁻ single bond ($\nu_{\text{imaginary}} = -41 \text{ cm}^{-1}$, RHF/6-31G*). In this conformation, the C–COO⁻ single bond is very long at 1.612 and 1.596 Å for the RHF and RHF/SCRf models, respectively. Using the SCRf aqueous solvation model and DFT, planar **3** was estimated to be 5.1 kcal/mol higher in energy than the twist minimum **4**, whereas the MP2 level calculation placed **3** only 0.9 kcal/mol higher in energy (Table 1). As for **4**, the carboxylate OCO angle of **3** is wide at 137.9° (RHF/6-31G*), but when solvation is included, a smaller value of 134.4° is predicted. Both the gas phase and solvated planar oxocarbenium ion models have slightly more double-bond character for the endocyclic oxocarbenium ion C2–O6 bond (1.249 and 1.242 Å) than predicted for the corresponding twist models (1.256 and 1.248 Å). Interestingly, in water, α -lactone **5** was estimated to be 7.8 kcal/mol higher in energy than the twist sialyl cation **4**, but was 3.7 kcal/mol more stable than **4** in the gas phase. At MP2 theory, this same trend was observed, but **5** was only 1.8 kcal/mol less stable than **4** in an aqueous solvation environment (Table 1).²⁴ RHF, DFT, and MP2 methods predict that the α -lactone **5** is favored in the gas phase but not in an aqueous environment. This change in the relative stability of **5** versus **3** and **4** in the gas and aqueous phases is due in large part to the preferential solute–solvent interactions for charged **3** and **4** in an aqueous medium. For example, when the solute–solvent interaction energy is compared for compounds **4** and **5**, it is found that **4** is stabilized by 9.4 kcal/mol over **5** (B3LYP/6-31G*/SCI-PCM).

Table 2 presents the changes in key geometric parameters that occur for conversion of the reactant state **6** to either the α -lactone **5**, planar cation **3**, or twist cation **4**. For the formation

of the planar cation **3**, the gas-phase results show a shortening of r_{C2-O6} and r_{C2-C3} , a lengthening of r_{C-COO} , and an increase in θ_{OCO} . The results for formation of the twist cation **4** are similar to those for the planar cation with the exception that r_{C-COO} decreases rather than increases. The parameters r_{C2-O6} and r_{C2-C3} are reflective of the oxocarbenium ion, the former providing a measure of O6 lone pair electrons' participation in stabilizing the positive charge at the former anomeric center C2 and the latter providing an indication of hyperconjugation between the C-3 hydrogens and C2; both phenomena contribute to a decrease in r_{C2-O6} and r_{C2-C3} bond lengths upon formation of the oxocarbenium ion. Electrostatic attraction between the electron-deficient oxocarbenium ion carbon and the negatively charged carboxylate oxygen atoms can account for the spread of the OCO bond angle upon oxocarbenium ion formation. Calculations which placed a positive point charge at varying distances from a carboxylate showed that, as the point charge was moved closer to the carboxylate, the OCO bond angle widened.

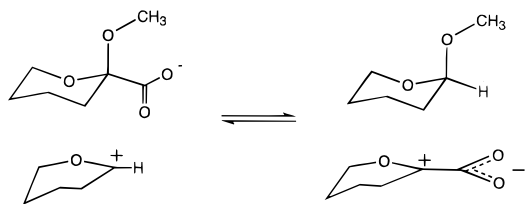
The effect of solvation may be compared to the gas-phase results (Table 2). For the planar oxocarbenium ion the changes for r_{C2-O6} and r_{C2-C3} were virtually identical to those predicted for the gas phase, but the increases in r_{C-COO} and θ_{OCO} were significantly smaller than those for the gas-phase process. For formation of the twist oxocarbenium ion **4**, solvation limited the increase in θ_{OCO} but did not affect the change in r_{C-COO} .

Because the acid-catalyzed solvolysis of CMP-NeuAc showed a large carbon KIE at the carboxylate, it was of interest to consider the effect of the carboxylate protonation state on molecular geometry and isotope effects. In aqueous solution the solvolysis of CMP-NeuAc could proceed with a number of interactions at the carboxylate group including hydrogen bonding, protonation, or ion pairing with alkali cations. All of these interactions can be considered to localize electron density at the carboxylate to varying degree, thus having possible importance regarding the isotope effects at the carboxylate carbon. Protonation can be considered to be an extreme form of bonding interaction that would result in electron localization at the carboxylate and so was chosen to provide a likely upper limit for effects derived from carboxylate bonding interactions. Optimized molecular geometries and frequencies have been calculated for the oxocarbenium ion in the protonated form (RHF/6-31G*/SCRf); the reactant state model **6** was also protonated (structure **7**, Figure 2), allowing comparison of the change in various geometric parameters in proceeding from reactant state to the oxocarbenium ion as a function of protonation (Table 2). The protonated cation optimized to a minimum energy geometry **8** (Figure 2), having a dihedral twist of 35.9° between the unprotonated carboxyl carbonyl oxygen and the endocyclic ring oxygen. The key effects of protonation

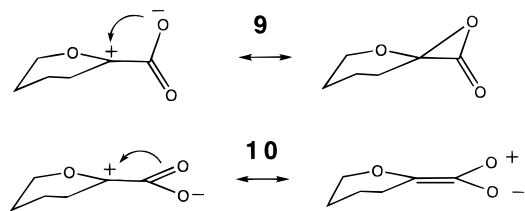
(24) In an *ab initio* study of the gas-phase internal S_N2 reaction of chloroacetate, it was found that only the α -lactone had a finite existence; the planar α -methylene zwitterionic species dissociated to CO₂ and methylene, and the twist conformer collapsed without barrier to the α -lactone. Antolovic, D.; Shiner, V. J.; Davidson, E. R. *J. Am. Chem. Soc.* **1988**, *110*, 1375–1381.

at the carboxylate of either the reactant or cation was to decrease θ_{OCO} and $r_{\text{C-COO}}$; however, the decrease in $r_{\text{C-COO}}$ for the cation was nearly twice that predicted for the reactant state (-0.061 and -0.032 Å, respectively).

Oxocarbenium Ion Stabilization by α -Carboxylate Substitution. It was anticipated that an α -carboxylate-substituted oxocarbenium ion would enjoy substantial electrostatic stabilization relative to an unsubstituted oxocarbenium ion. An estimate of the stabilization is obtained by analysis of the isodesmic reaction shown below. In the gas phase, an α -carboxylate-substituted oxocarbenium ion is predicted to be 110 kcal/mol more stable than the H-substituted analogue. The results obtained for aqueous solvation predict that the sialyl cation is favored over the α -H-substituted oxocarbenium ion by 17 kcal/mol. These results point to the greater thermodynamic stability of α -carboxylate-substituted oxocarbenium ions over H-substituted analogues, but a reliable estimate of their relative lifetimes cannot yet be made in the absence of further information, especially given the observation that kinetic and thermodynamic stability of carbenium ions do not necessarily correlate.²⁵ The large drop from 110 kcal/mol in the gas phase to 17 kcal/mol confirms that the source of the stabilization is primarily electrostatic in nature, but it was of interest to consider covalent



interactions as well. A small component of the stabilization could arise from covalent interactions such as electron donation from a carboxylate oxygen into the electron-deficient oxocarbenium carbon p_z orbital for the twist sialyl cation as in **9** or involve π -type delocalization between the carboxylate carbonyl and the carbon p_z orbital for the planar sialyl cation as in **10**.²⁶



The Mulliken overlap populations for the twist and planar sialyl cations support the existence of a small degree of covalent interactions of the sort shown in **9** and **10**. Approximately $0.06 e^-$ are shared between the carboxylate oxygens and the carbon p_z orbitals for the twist sialyl cation. This additional electron density in the carbon p_z orbital would be consistent with the calculated β - ^2H isotope effects for the twist isomer (discussed below), which are lower than those predicted for the planar isomer, and bonding as in **9** would tend to open the carboxylate OCO bond angle. As will be discussed below, however, the calculated isotope effects for the twist sialyl cation model **4** are not in agreement with the experimental results. For the planar cation, $0.02 e^-$ is shared between the π -symmetric oxocarbenium ion and carboxylate carbon p_z orbitals. That this is not a significant component of binding is clear, since a shortening of

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(26) Creary, X.; Wang, Y.-X.; Jiang, Z. *J. Am. Chem. Soc.* **1995**, *117*, 3044–3053.

the C–COO bond would be predicted, contrary to the results of the calculations (Table 2).²⁷

Isotope Effects. The magnitude of the experimental KIE's for the acid-catalyzed solvolysis of CMP-NeuAc indicated that the transition state is very late, approaching a fully formed oxocarbenium ion.⁵ A good match between the experimental KIE's and calculated equilibrium isotope effects for formation of the oxocarbenium ion should provide a good description of the transition state structure for the solvolytic reaction. Table 2 presents the calculated equilibrium isotope effects for conversion of reactant state **6** or **7** (protonated) to the α -lactone **5**, planar oxocarbenium ions **3** and **8** (protonated), or twist oxocarbenium ion **4**. For comparison, the experimental kinetic isotope effects for solvolysis of CMP-NeuAc are presented at the top of the table. Results for the 6-31G* basis are shown; 6-31G** and 6-31+G* bases and inclusion of solvation did not have a significant effect on calculated isotope effects.²⁸

The best match to the experimental isotope effects is found for the planar oxocarbenium ion **3**; the twist oxocarbenium ion **4** is predicted to have diminished carbon and deuterium isotope effects with values well outside the generally good match found for the planar model. The large experimental β - ^2H KIE is inconsistent with a transition state strongly resembling α -lactone **5**, for which near-unity β - ^2H isotope effects are predicted. Similarly, calculated β - ^2H isotope effects for formation of twist oxocarbenium ion **4** are significantly lower than the experimental value. The diminished values of the β - ^2H isotope effects for **4** and **5** are a reflection of bonding interactions at the anomeric carbon C-2 which reduce hyperconjugation. On this point it is interesting to note that the small β - ^2H KIE's reported for the water-catalyzed solvolysis of aryl glycosides of *N*-acetylneuraminic acid have been rationalized on the basis of α -lactone transition state character.²⁹

The calculated ^{14}C isotope effects at the anomeric carbon C-2 are below the experimental range for the twist cation **4** and the α -lactone **5**, whereas the predicted value for the planar cation **3** is in agreement with experiment. The agreement for **3** but not **4** and **5** may be attributed to a net loosening at the anomeric carbon upon scission of the glycosidic bond, which in the case of **4** and **5** is offset by additional bonding interaction between a carboxylate oxygen and C-2.

At the onset of this work it was suspected that the carboxylate ^{14}C isotope effect for formation of an α -lactone might be inverse or near unity due to strain. This was not the case, however, as the calculations predict isotope effects equivalent in magnitude to those predicted for formation of the twist sialyl cation but smaller than those predicted for formation of the planar sialyl cation. Again, the best match between theory and experiment is for formation of the planar oxocarbenium ion **3**.

Carbon-14 Fractionation Factors for Protonation of the Carboxylate. It came as a surprise that the carboxylate protonation state could significantly influence the size of the carboxylate ^{14}C isotope effect (Table 2), since the experimental fractionation factor for ionization of a carboxylic acid was expected to be close to unity.³⁰ When the carboxylate ^{14}C

(27) Inclusion of electron correlation (single point, B3LYP/6-31G*) showed an increase in π character to $0.05 e^-$ between the oxocarbenium ion carbon and the carboxylate carbon of the planar sialyloxocarbenium ion, mirroring the trend reported by Creary et al.²⁶ for a formylcarbenium ion.

(28) A similar observation has recently been reported for solvation effects on vibrational frequencies: Williams, R. W.; Cheh, J. L.; Lowrey, A. H.; Weir, A. F. *J. Phys. Chem.* **1995**, *99*, 5299–5307.

(29) Ashwell, M.; Guo, X.; Sinnott, M. L. *J. Am. Chem. Soc.* **1992**, *114*, 10158–10166.

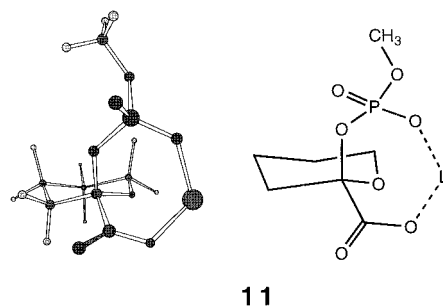
(30) Bayles, J. W.; Bron, J.; Paul, S. O. *J. Chem. Soc., Faraday Trans. 1* **1976**, *7*, 1546–1542.

fractionation factor was calculated for ionization of this group in the reactant **6** ($7 \rightarrow 6$), it was found to be ~ 1.006 , in agreement with precedent; however, the fractionation factor for the planar oxocarbenium ion **3** ($8 \rightarrow 3$) was calculated to be ~ 1.025 . The fractionation factor for the carboxylate protonation state will be superimposed on any isotope effect associated with forming the sialyl cation, if the ionization state of the carboxylate group changes during the reaction. Conversion of unprotonated reactant **6** to protonated oxocarbenium ion **8** has a small ^{14}C isotope effect because the fractionation factor for protonating the carboxylate makes an inverse contribution to the normal isotope effect for formation of the oxocarbenium ion; conversion of protonated reactant **7** to unprotonated **3** has a slightly larger isotope effect than that for $6 \rightarrow 3$ because the fractionation factor for the reactant state reinforces the isotope effect for formation of the oxocarbenium ion.³¹

The experimental isotope effects are in good agreement with those predicted for conversion of the protonated reactant **7** to the planar oxocarbenium ion with an ionized carboxylate **3** (Table 2) and for conversion of the ionized reactant **6** to the planar, ionized oxocarbenium ion **3**. Models which involved a protonated oxocarbenium ion had predicted isotope effects which were quite inconsistent with the experimental data. The solution $\text{p}K_a$ of the CMP-NeuAc carboxylate group has been measured titrimetrically to be less than 4, and a kinetic $\text{p}K_a$ of ~ 4 was estimated from the pH–rate profile for solvolysis of CMP-NeuAc.⁵ Since the kinetic isotope effect data were obtained at a pH of 5.0, the dominant reaction manifold for solvolysis involves an ionized carboxylate group. One way in which to reconcile these observations would be if the reactant state (CMP-NeuAc) carboxylate is not protonated, but rather is either hydrogen bonded to water and/or ion paired with sodium counterions. On proceeding to the transition state, disruption of this bonding scheme may have an influence on the isotope effects in a way which is similar to ionization of a protonated carboxylate. Though presently speculation, such a desolvation might enhance carboxylate stabilization of the oxocarbenium ion transition state. Carboxylate desolvation has been proposed to be involved in solvolysis of aryl glycosides of *N*-acetylneuraminic acid, though in that case the desolvation was suggested to precede α -lactone formation.²⁹

In summary, the calculated equilibrium isotope effects for formation of the planar oxocarbenium ion **3** are in very good agreement with the experimental kinetic isotope effects for acid-catalyzed solvolysis of **1**, while poor agreement is found for the twist oxocarbenium ion **4** and α -lactone **5**. A conservative description of the solvolytic transition state would feature a nearly fully formed oxocarbenium ion with the carboxylate group sufficiently close to planarity as to account for the isotope effects despite any possible energetic penalty for residing in the planar conformation. It has been proposed that the solvolysis of aryl glycosides of *N*-acetylneuraminic acid involves a nucleophilic carboxylate group which leads to a transition state with α -lactone character, necessitating, in that case, a twisted conformation for the carboxylate group.²⁹ One possible explanation for this divergent behavior would include the ~ 1000 -fold lower reactivity of aryl leaving groups versus cytidine monophosphate; departure of the poorer aryl leaving group may be assisted by nucleophilic participation. Another possibility might be coordination of the NeuAc carboxylate with the CMP phosphate via a hydrogen bond or alkali cation bridge; such an interaction is not possible for aryl glycosides of *N*-acetylneuraminic acid. A MOPAC6/MNDO geometry optimization

for the lithium chelate **11** placed the carboxylate at a twist angle of $\sim 21^\circ$ between one of the carboxylate oxygens and the endocyclic oxygen of NeuAc. This structure places the carboxylate near planarity to the incipient oxocarbenium ion plane.



Correlation of the Carboxylate ^{14}C Isotope Effect with Geometric and Vibrational Parameters. Solvolysis of CMP-NeuAc proceeds with a ^{14}C KIE of 1.036 at the carboxylate carbon, a value considerably larger than typical secondary carbon isotope effects. A normal isotope effect will arise if the bonding about the carboxylate becomes looser in the sialyl cation than for the reactant state. A comparison of the changes in bond length and angles to the carboxylate group ($\Delta r_{\text{C2-COO}}$ and $\Delta \theta_{\text{OCO}}$ columns, Table 2) with the calculated carboxylate ^{14}C isotope effects (1- ^{14}C column, Table 2) reveals that small values for the isotope effect are associated with small changes in bonding to the carboxylate (little or no loosening), whereas the largest isotope effects are associated with large changes in carboxylate bonding. This correlation then predicts that, in proceeding from the reactant state to the sialyl cation, isotopically sensitive carboxylate vibrational modes should shift to lower frequency, and this was found to be the case after inspection of the frequencies calculated with the QUIVER program.²³ The biggest contributors are coupled vibrations that involve the C–COO stretch and OCO angle bending mode; this description is somewhat simplified in that these modes are also coupled in varying degree to ring stretches and bends. The asymmetric carboxylate C–O stretch is 117 cm^{-1} higher in frequency for the planar sialyl cation than for the reactant providing an inverse contribution to the overall normal isotope effect.

From this analysis it is concluded that the experimentally observed secondary ^{14}C kinetic isotope effect is well modeled by an overall loosening of the forces at the carboxylate carbon in the transition state relative to the reactant state. The looser carboxylate environment can be accounted for by formation of the oxocarbenium ion, which draws the oxygens of the carboxylate toward it by electrostatic interaction, opening the OCO bond angle.

Conclusions. Oxocarbenium ions with an α -carboxylate substituent are substantially stabilized relative to the H-substituted oxocarbenium ion by electrostatic interaction. The origin of the unusually large carboxylate ^{14}C isotope effect for solvolysis of CMP-NeuAc is attributed to an electrostatically driven opening of the carboxylate OCO bond angle upon formation of the sialyl cation. Given the predicted sensitivity of carboxylate ^{14}C isotope effects to the carboxylate bonding scheme and electrostatic environment, carboxylate carbon isotope effects may be useful as a sensitive probe for a number of enzymatic systems including sialyltransferases, neuraminidases, KDO-8-phosphate synthase, and UDP-GlcNAc enolpyruvyl transferase.

The experimental isotope effects for solvolysis of CMP-NeuAc are in very good agreement with a transition state model involving formation of a planar or near-planar sialyl cation with

(31) Fractionation factors for $2\text{-}^{14}\text{C}$ substituted **6** and cation **3** as a function of carboxylate protonation state have also been calculated and account for the variation of the $2\text{-}^{14}\text{C}$ isotope effect shown in Table 2.

an ionized carboxylate group. In water, the twist oxocarbenium ion **4** is estimated to be ~ 0.4 – 5.1 kcal/mol more stable than the planar oxocarbenium ion **3**, which is a transition structure with respect to rotation about the C–COO bond at the 6-31G* RHF level. Since SCRF solvation models assume nonspecific interactions between solute and solvent, the relative energetics do not consider specific solvation phenomena such as hydrogen bonding, which will be the subject of further investigations. However, the results taken together suggest that, while the transition state for formation of the sialyloxocarbenium ion involves a near-planar relationship between the carboxylate and oxocarbenium ion atoms, progress to an oxocarbenium ion intermediate may involve rotation of the carboxylate to the energetically favored twist conformation. Rapid attack by solvent on this species from either face of the oxocarbenium ion plane could account for the experimentally observed racemization in the product.⁵ As an alternative, collapse of the twist oxocarbenium ion to give a mixture of epimeric α -lactones which subsequently solvolyze to racemized product is less

attractive, since α -lactone formation is estimated to be endothermic by ~ 2 – 10 kcal/mol in an aqueous environment.

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Supporting Information Available: Tables of Cartesian coordinates for all models and frequencies for $^{12}\text{C}/^{14}\text{C}$ -substituted reactant **6** and oxocarbenium ion **3** (11 pages). See any current masthead page for ordering and Internet access instructions.

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