in these bulk samples has been suggested to affect PL intensity by changing the depletion region width and/or the surface recombination velocity. All solvents that we have investigated *reduce* the emission intensity of porous Si, indicating that the solvent interactions increase nonradiative recombination in this particular material. An interpretation consistent with the observed data is that the dipoles attract either electrons or holes to surface traps. On very small particles, the carrier recombination rate should be dependent on the product of the electron and hole concentrations (the number of dopant atoms present in a 10-nm diameter piece of 0.642 Ω-cm n-Si is less than 1, so in a photoexcited nanoparticle the carrier concentrations are comparable). Thus, trapping of either electrons or holes at the surface should reduce luminescence intensity. Although this interpretation explains the correlation to solvent dipole moment, the observation that nonpolar molecules such as benzene also cause significant PL quenching indicates that the above interpretation is overly simplified. In addition, the fact that there is no detectable PL quenching on exposure to water vapor is surprising. This may be due to the inability of $H_2O$ to preferentially align on the hydrophobic hydrogen-terminated Si surface. Current experiments are aimed at understanding the mechanism of PL quenching in more detail. The extreme sensitivity of the luminescent porous Si surface presents the potential for application of this material in chemical sensors.

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## Aqueous Basicity of the Carboxylate Lone Pairs and the C–O Barrier in Acetic Acid: A Combined Quantum and Statistical Mechanical Study

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The stereoelectric effects at carboxyl oxygen are of importance in the understanding of the mechanism of organic and enzymatic reactions as well as molecular recognition processes.[1] Consequently, there has been a continuous effort in both theoretical and experimental investigations.[2-8] Gandour pointed out 10 years ago that the syn-oriented carboxylate is preferred in enzymatic catalysis because of its much higher basicity than the anti form.[2]

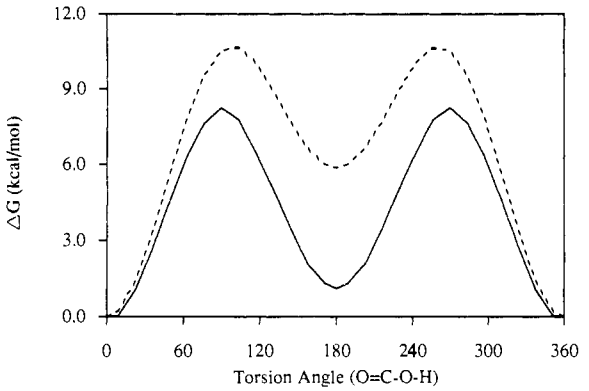


**Figure 1**. Computed rotational energy function (- - -) and potential of mean force in aqueous solution (—) for $CH_3COOH$ at 25 °C. Torsional angle is in degrees.

However, recent studies of model systems indicate that there is only minimal syn-stabilization in these compounds in solution.[3,4] These effects have also been thoroughly investigated by Houk and Wiberg and their co-workers using ab initio molecular orbital methods.[6,7] Nonetheless, little is known about the solvent contribution to this phenomenon.[8b] To assess the solvent effects on the relative basicity of carboxylate lone pairs in water, we have carried out Monte Carlo simulations using a combined quantum mechanical (QM) and molecular mechanical (MM) potential to compute the potential of mean force (pmf) of the C–O rotation in acetic acid. Although such potentials have been applied in energy minimization and relative free energy calculations,[9-11] they have not been used for determination of a complete pmf in solution.

The combined QM/MM potential for a condensed-phase system as defined by Field et al.[10] is partitioned into a QM region consisting of the solute and an MM region of solvent molecules. The total energy of the system is obtained from the QM particle electronic Hamiltonian ($\hat{H}_{QM}$), the QM/MM interaction Hamiltonian ($\hat{H}_{QM/MM}$), and MM potential functions,[10,11] while the pmf is determined via statistical perturbation theory (eq 1) by computing free energy changes along the reaction coordinate,[12] $\theta$;

$$\Delta G(\theta_0 \rightarrow \theta_1) = -kT \ln \langle e^{-\Delta E(\theta_0 \rightarrow \theta_1)/kT} \rangle_0 \quad (1)$$

where $\langle\ \rangle_0$ represents the ensemble average with the Hamiltonian $\hat{H}(\theta_0)$, and $\Delta E(\theta_0 \rightarrow \theta_1)$ is the energy difference between states $\theta_0$ and $\theta_1$. $\Delta E(\theta_0 \rightarrow \theta_1)$ is given by eq 2 and is evaluated using the method described previously:[11]

$$\Delta E(\theta_0 \rightarrow \theta_1) = \Delta E_{pol}(\hat{H}_{QM}[\theta_0 \rightarrow \theta_1]) + \Delta E_{1or}(\theta_0 \rightarrow \theta_1) + \Delta E(\hat{H}_{QM/MM}[\theta_0 \rightarrow \theta_1]) + \Delta E^{vdW}_{QM/MM}(\theta_0 \rightarrow \theta_1) \quad (2)$$

Here, the terms on the right-hand side of eq 2 are, respectively, differences in solute polarization, gas-phase torsional energy, and solute–solvent electronic and solute–solvent van der Waals energies.[11] The electric polarization energy for the solute is defined as follows:

$$E_{pol}(\hat{H}_{QM}(\theta)) = \langle \Phi | \hat{H}_{QM}(\theta) | \Phi \rangle - \langle \Phi^\circ | \hat{H}_{QM}(\theta) | \Phi^\circ \rangle \quad (3)$$

where $\Phi^\circ$ and $\Phi$ are, respectively, the wave functions for QM particles in the gas phase and in aqueous solution. Applying eq

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0002-7863/92/1514-1912$03.00/0 

4,[13,14] the free energy profile can further be decomposed into enthalpic ($\Delta H$) and entropic ($\Delta S$) contributions,

$$-T\Delta S(\theta_0 \rightarrow \theta_1) = \Delta G(\theta_0 \rightarrow \theta_1) - \left[ \frac{\langle H(\theta_1) e^{-\Delta E/kT} \rangle_0}{\langle e^{-\Delta E/kT} \rangle_0} - \langle H(\theta_0) \rangle_0 \right] \quad (4)$$

where $H(\theta_0)$ and $H(\theta_1)$ are the total enthalpies of the system for the reference and perturbed states. For acetic acid, the reaction coordinate, $\theta$, is chosen as the O=C—O—H dihedral angle that varies from 0° (syn) to 180° (anti).

The pmf for the O=C—O—H rotation in water was obtained through Monte Carlo simulations in the NPT ensemble at 25 °C and 1 atm.[14] A cubic cell containing 216 water molecules plus an acetic acid molecule was used in all computations. Periodic boundary conditions along with a cutoff distance of 9 Å were employed to evaluate the interaction energies. In the fluid simulation, $CH_3COOH$ was treated quantum mechanically using the AM1 model,[15] while the TIP3P model was adopted for water.[16] The computationally efficient AM1 method enables the QM energy to be computed throughout the Monte Carlo simulations.[11] Furthermore, bond lengths and angles of the solute were optimized whenever there was a solute move during the Metropolis sampling. Geometry optimizations were needed because the methyl rotation in acetic acid was sampled over in the simulation through dihedral variation,[14] whose value was kept fixed in the AM1 optimization. However, the optimization excludes the solute–solvent interaction Hamiltonian, so that the solute structure corresponds to that in the gas phase. The solute–solvent interaction energy was obtained by a single-point SCF calculation including $\hat{H}_{QM/MM}(\theta)$.[11] Statistical perturbation computations were performed with double-wide sampling and $\Delta\theta$ values of ±9°.[17,18] Each simulation involved at least 500K configurations for equilibration and 1.5M configurations for averaging, which took approximately 3.5 days with one processor on a Stardent 3030 computer.

Figure 1 shows the key results of the present study. The AM1 model predicts that the anti conformation in $CH_3COOH$ is 5.9 kcal/mol higher in energy than the syn in the gas phase, which agrees exactly with the best ab initio estimate at the MP3/6-311+G(pd) level.[6,7,19] The computed free energy difference between the two conformers is reduced to 1.1 ± 0.3 kcal/mol in aqueous solution, in good accord with Rebek's experimental estimate of 1–2 kcal/mol.[3] For comparison, the difference in solvation free energy was computed to be 6.3 ± 0.3 kcal/mol using the OPLS potentials in favor of the anti conformation. This translates to an anti–syn free energy difference of −0.4 ± 0.3 kcal/mol in water.[19] Thus, it appears to be necessary to modify the OPLS charges for (*E*)-acetic acid as in the case of *N*-methylacetamide.[18] Given the fact that this free energy difference is a direct measure of the relative basicity of the carboxylate syn and anti lone pairs, our results suggest that the diminished syn-stabilization observed in the model compounds for serine proteases, intramolecular general base catalysis, and the carboxylate–imidazole pair is due to the solvent effects,[3,4] though distortions from ideality in these molecules may also contribute.[3c,7b] The preferred syn orientation in the His–Asp couple found in many enzymes is likely the result of efficient packing arrangement.[2] To ensure the validity of the AM1/TIP3P model used in the present simulations, bimolecular complexes were studied, treating $CH_3COOH$ quantum mechanically and $H_2O$ molecular mechanically.[10,11] The results are compared with those obtained from ab initio 6-31G(d) and 6-31+G(d) calculations (supplementary material).[8b] The agreement for H-bond complexes involving the carbonyl group is excellent, whereas hydrogen bonding with the hydroxyl group is different by 1–2 kcal/mol between the ab initio and AM1/TIP3P results. However, the major concern is whether there is a net bias for hydration of either the syn or anti conformers.[18] From the comparison, the overall bias for the trans isomer in the eight structures considered was found to be only 0.2 kcal/mol. Consequently, this provides a further support to the computed results.

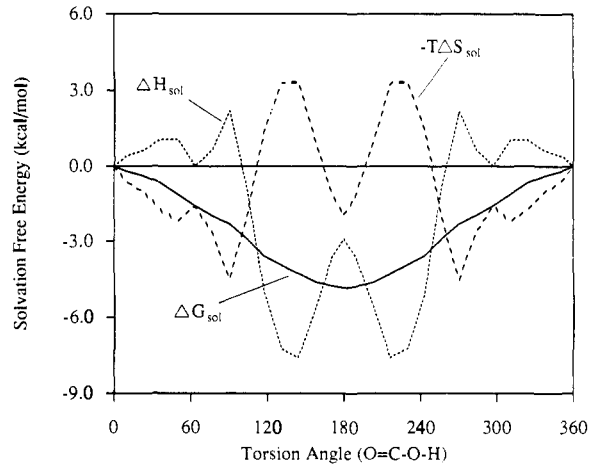


**Figure 2.** Computed solvation free energy, enthalpy, and entropy for the C–O rotation of acetic acid in water. Torsional angle is in degrees.

The origin of the rotational barrier in acetic acid in the gas phase has been characterized by Wiberg and Laidig.[6] For comparison, the AM1 value is 2 kcal/mol smaller than the best ab initio prediction (12.6 kcal/mol).[6a] Our simulation yields a barrier height of 8.3 ± 0.2 kcal/mol in aqueous solution, a drop of 2.3 kcal/mol from the gas-phase value. Examination of the solvation energy profiles shown in Figure 2 reveals that the solvent-induced reduction of barrier height is mainly due to the favorable entropic contribution, while the enthalpy of solvation actually raises the energy by ca. 2 kcal/mol.[13] In the anti conformation region, the solvation enthalpy becomes more favorable, being the largest at a dihedral angle of 145°. Figure 2 also shows that the difference in free energy of solvation between the syn and anti conformers results from both enthalpic and entropic effects; however, the enthalpy contribution is about 1 kcal/mol greater than the entropic. Another interesting result is the electronic polarization energy of the solute owing to the interaction with solvent molecules; the value continuously increases from 1.3 ± 0.1 in the syn conformer to 3.7 ± 0.3 kcal/mol in the anti configuration. This can be rationalized as the result of a stronger solvation of the anti geometry in water due to its much larger dipole moment than the syn conformation (AM1, 1.89 D vs 4.39 D; 6-31+G(d), 1.94 D vs 4.97 D).[20,21]

**Supplementary Material Available:** Details of the geometrical and energetic results of the biomolecular complexes computed

(13) This formula was derived in 1988 during a CECAM workshop on free energy calculations at Orsay, France; Gao, J.; Karplus, M. Unpublished results. It was also programmed by Professor Jorgensen in his BOSS program. Like other methods, the hysteresis associated with the calculated entropy was too large to be of general use; however, it could also provide meaningful interpretations for well-designed systems. In the present study, the cumulative uncertainties ($\pm 1\sigma$) are ca. ±1.4 kcal/mol at the ends of the pmf for both enthalpy and entropy ($T\Delta S$) contributions if the average starts in the middle. See also: (a) Roux, B.; Karplus, M. *Biophys. J.* **1991**, *59*, 961. (b) Roux, B. Ph.D. Thesis, Harvard University, Cambridge, MA, 1990.

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(19) The experimental *E*/*Z* energy difference for acetic acid did not appear to be available from the literature. However, Raman and neutron diffraction studies yielded values of 3.90 and 4.78 kcal/mol for formic acid (ref 8b), which may be compared with the computed result of 4.6 kcal/mol at the MP3/6-311+G(dp) level (ref 6). Therefore, it seems that the *E*/*Z* energy difference predicted for acetic acid at the same theoretical level should be reasonable.

(20) Gratitude is expressed to the donors of the Petroleum Research Fund, administered by the American Chemical Society, the NIH for a Biomedical Research Support Grant, and SUNY at Buffalo for support. The authors also wish to thank Professors Jorgensen and Truhlar for discussion on the solvation free energy obtained from the OPLS potentials.

(21) Supplementary material.

using the AM1/TIP3P model and ab initio method in Z-matrix format (4 pages). Ordering information is given on any current masthead page.

## Internal Hydrogen Abstraction by Activated Neocarzinostatin: Quenching of the Radical at C2 by Hydrogen Atom Transfer from the α Carbon of the Adducted Thiol

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Neocarzinostatin chromophore (NCS-Chrom) (**1**) (Scheme I) is the first of the enediyne-containing antitumor antibiotics shown to cleave DNA by abstracting hydrogen atoms from minor groove accessible sites on the deoxyribose backbone.[1] Thiol adduction at C12 of the bicyclo enediyne ring generates a diradical species of the drug, with radical centers at C2 and C6 (**3**), that eventuates in a stable reduced form of the drug (**4**) following hydrogen atom abstraction from DNA or some other available source.[2-5] Although the sulfhydryl hydrogen can quench the radicals of the activated chromophore at high ratios of thiol to drug,[6] under the experimental conditions used in DNA damage reactions involving relatively low ratios of thiol to drug, hydrogen from the exchangeable sulfhydryl, as well as that from the nonexchangeable DNA source, cannot account for the total hydrogen abstracted into the post-activated thiol-drug adduct.[3-5] Hence, the acidic hydrogens on the carbon α to the sulfur in the thiol have been suggested as a possible source of hydrogen abstracted by the diradical form of NCS-Chrom.[4] We present herein direct evidence of hydrogen atom abstraction from the carbon α to the sulfur of a drug-bound thiol into the C2 position of the drug. Intramolecular quenching of the radical at C2 helps to explain the finding that single-stranded DNA breaks exceed double-stranded lesions.[1]

Since glutathione activates NCS-Chrom in cells,[7] it was the thiol chosen in this study to investigate the hydrogen abstraction reaction. A racemic mixture of γ-L-glutamyl-DL-cysteinylglycine (DL-**2**), with or without deuterium replacing both hydrogens on the α carbon, was prepared by solid-phase peptide synthesis. DL-Cysteine was converted[8-10] into *N*-Fmoc-*S*-trityl-DL-cysteine for peptide synthesis. $^1$H NMR analysis showed that the deuterium enrichment of the α carbon was more than 90%. The ability of DL-**2** to activate NCS-Chrom was verified by measuring the incorporation of tritium into the drug from [5′-$^3$H]thymidine-labeled λ DNA[4] at a thiol to drug ratio of 5. DL-**2** affords the same amount of tritium abstraction from DNA into the drug as glutathione. The thiol-drug adducts (**4**) were produced by mixing the thiol with NCS-Chrom[4] at a ratio of 8, using sodium citrate and Tris-HCl as buffers. In the absence of DNA, reactions were conducted at a temperature range from 0 to 20 °C, a pH range from 4 to 8.5, and with methanol at 1 to nearly 100%. In reactions involving calf thymus DNA, the ratio of DNA phosphorus to drug was 10.

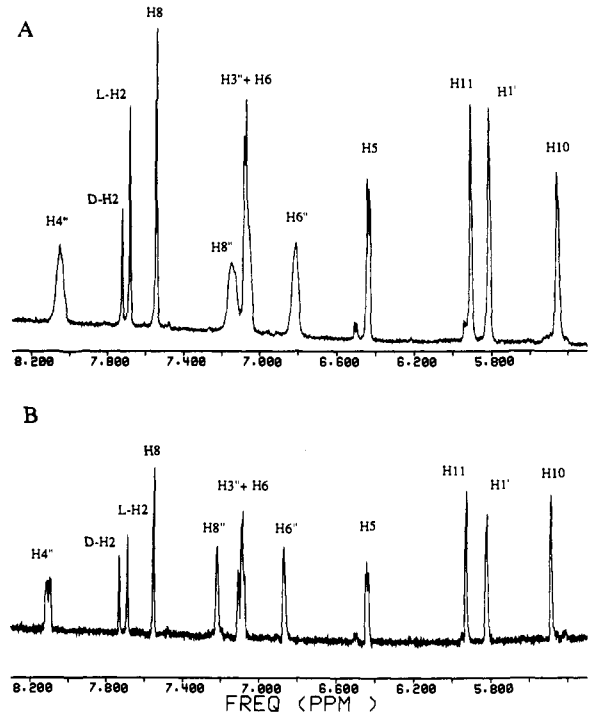
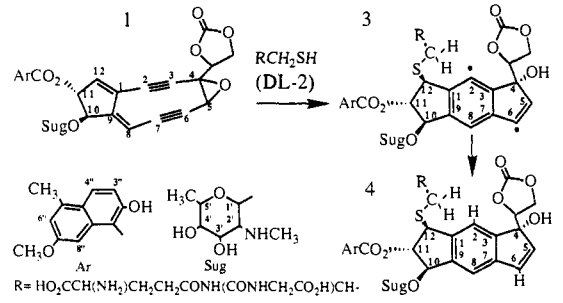



**Figure 1.** $^1$H NMR spectra (500 MHz) (δ 5.3-8.3) of purified thiol-drug adducts produced by mixing thiol and NCS-Chrom (8:1) at pH 7.6 in a >99% methanolic solution at 20 °C: DL-**2**-NCS-Chrom adduct (A); [α,α-$^2H_2$]-DL-**2**-NCS-Chrom adduct (B). The NMR solvent was 2 mM $^2$HCl in $^2H_2O$.

**Scheme I**




Analysis of the 500-MHz $^1$H NMR spectra of the DL-**2**-drug adduct (Figure 1) revealed that the majority of the resonance signals from the rearranged central core of the post-activated drug exhibited close, if not identical, chemical shifts for the D and L forms of the compound. The signal for the D form of H2, however, was well-separated from that of the L form by 0.05 ppm downfield. Analysis of the spectra of the DL-**2**-drug and [α,α-$^2H_2$]-DL-**2**-drug adducts from paired experiments showed that 20-30% of deuterium was consistently incorporated into C2 of the L-form adduct (seven sets of experiments, two with DNA) under the various conditions. There was no obvious reduction of the signal at H2 of the D-form adduct or at H6 in the spectra of the [α,α-$^2H_2$]-DL-**2**-drug adduct. In fact, the H2 peak of the D-form adduct is slightly greater than that of the D form of the nonlabeled adduct. The amount of deuterium transfer to C2 is not very sensitive to changes in temperature, percent of methanol, pH, or the presence of DNA. However, the yield of the two isomeric adducts, as judged from the separate $^1$H NMR signals at H2, varies substantially with the content of methanol and the presence of DNA.

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