Axial selectivity with **2d** is the result of unusually slow equatorial attack. Thus, a 1:1 mixture of equatorial **1c** and axial **2d** was subjected to osmylation with 0.1 equiv of OsO_4/pyridine. Diols derived from **2d** could not be detected by NMR analysis of the product (≥20:1 **8c**:**9d**). Since **9d** is formed ca. seven times faster than **10d** (Table II), the axial CH_3O group decreases the rate of *equatorial* osmylation of **2d** by at least two orders of magnitude relative to **1c**. Axial attack on **2d** is retarded less, and this is the source of the selectivity.

The trend for avoidance of ether oxygen is smaller when conformational restrictions are imposed on the C–O bond. Spirocyclic ethers **11** and **12** (prepared by Mitsunobu cyclization of the corresponding diols)[6] were subjected to catalytic osmylation. Isomer **11** was unexceptional and gave the normal preference for equatorial attack (ca. 4:1 **14**:**13**, >90%),[6] but **12** reacted nonselectively and afforded a 1.1:1 mixture of diols (>90%). Higher selectivity in the unconstrained methoxy analogues **2** is therefore related to alkoxy rotamer issues and bears no simple relationship to the nature of anti σ orbitals.

The epoxidations of **1a–e** and **2d,e** are qualitatively consistent with a variation of the torsional explanation of Cherest and Felkin.[4a] The developing C_1- -O bond must be longer than the partially rehybridized exocyclic methylene bond in the transition state (TS), and the latter should make the dominant contribution to the *steric component* (repulsion of filled orbitals) of torsional effects (1,2-interactions).[14] Comparison of *reactant-like* TS geometries shows that equatorial bonding encounters substantial eclipsing interactions, while axial bonding results in a more staggered arrangement of adjacent bonds. The result is an advantage for the axial product, provided that the reagent can avoid 1,3-interactions with the axial C–H bonds. This requirement is easily met in the epoxidations because asynchronous bonding of oxygen should be advanced at the methylene terminus vs C_1, placing the reagent far from cyclohexane substituents. The other axially selective additions of **1a** can be interpreted in a similar way by comparing 1,2-interactions of existing and developing bonds.[1,2] Caution is recommended because the torsional effect depends on the degree of rehybridization and on other details of TS geometry, but the concept is useful when the reagents are compact.

In the osmylations, the TS should be more product-like, and both the equatorial and axial bonding modes would have staggered geometries. Under these circumstances, 1,3-interactions due to osmate ligands can play the dominant role, and the equatorial TS is favored.[15] However, this trend is easily overcome by the oxygen avoidance phenomenon mentioned earlier.

Torsional barriers are proposed to contain a substantial σ,σ^* component as well as components due to filled orbital interactions.[14a] Their relative importance in the ground state is controversial,[14] but Tables I and II show no TS correlation between donor–acceptor properties of axial substituents and epoxidation or osmylation stereochemistry. The σ,σ^* contribution to $\Delta\Delta G^*$ must therefore be small. Hyperconjugative interactions may control stereochemistry in the absence of steric bias,[16] but other variables become more important in typical substrates, especially when heteroatoms are present.

The early TS concept of dominant 1,2-interactions by the existing bond vs the developing bond provides a simple explanation for other stereochemical results, such as the preferred axial addition of compact nucleophiles to cyclohexanones.[17] Solvation of the partial negative charge at oxygen (or coordination by bulky Lewis acids)[17b] may increase the effective bulk of the existing C–O bond and would further destabilize a partially eclipsed early TS for equatorial bonding.

Our discussion emphasizes repulsive terms of the torsional effect (steric repulsions, etc). Further investigations are under way to clarify the magnitude and geometric dependence of the 1,2- vs 1,3-interactions.

Acknowledgment. This work was supported by the National Science Foundation.

Supplementary Material Available: Spectral data (R_f, MS, IR, and ^{13}C NMR) for **1c–f**, **2c–g**, **11**, **12**, **3c–f**, **4c–e**, **5d,e**, **6d,e** and acetonides of **7c,d**, **8b–e**, **9c–g**, **10c–g**, and **13** (8 pages). Ordering information is given on any current masthead page.

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Kinetics of the Reaction of β-Methoxy-α-nitrostilbene with Thiolate Ions. First Direct Observation of the Intermediate in a Nucleophilic Vinylic Substitution

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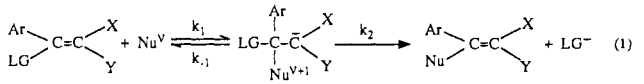
Nucleophilic vinylic substitution on substrates such as **1**, where XY are strongly electron-withdrawing groups and LG is a relatively sluggish leaving group, is believed to proceed by the addition–elimination mechanism[1] shown in eq 1. The evidence



(1)

1 2 3

includes the observation of base catalysis with amine nucleophiles,[2] k_{Br}/k_{Cl} ratios close to unity and k_F/k_{Cl} ratios ≫ 1[1a,d,e] (Br, Cl, F = LG), and the observation of stereoconvergence[1d,3] (both E and Z substitution products formed starting from either E or Z precursor). We now report the first example where **2** can be directly observed under conditions conducive to substitution. The

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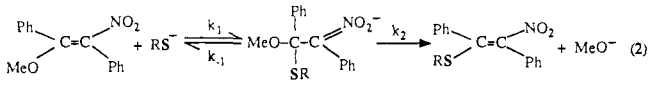
Table I. Rate and Equilibrium Constants of the Reaction of Thiolate Ions with (*E*)-β-Methoxy-α-nitrostilbene in 50% Me_2SO–50% Water at 20 °C[a]

	EtS^-	$HOCH_2CH_2S^-$	$MeO_2CCH_2CH_2S^-$	$MeO_2CCH_2S^-$
pK_a^{RSH} [b]	11.26	10.56	10.40	8.83
k_1, M^{-1} s^{-1}	(4.21 ± 0.20) × 10^2	(3.90 ± 0.19) × 10^2	(3.60 ± 0.16) × 10^2	(2.60 ± 0.04) × 10^2
k_{-1}, s^{-1}	(7.85 ± 1.73) × 10^{-3}	(5.10 ± 1.00) × 10^{-2}	(8.99 ± 1.90) × 10^{-2}	(5.38 ± 0.10) × 10^{-1}
$K_1 = k_1/k_{-1}$, M^{-1}	(5.36 ± 1.21) × 10^4	(7.65 ± 1.55) × 10^3	(4.00 ± 0.90) × 10^3	(4.83 ± 0.12) × 10^2
k_2, s^{-1}		(9.6 ± 1.5) × 10^{-6}		

[a] Ionic strength 0.5 M (KCl). [b] Measured in 50% Me_2SO–50% water.

reaction is that of alkylthiolate ions with (*E*)-β-methoxy-α-nitrostilbene (eq 2)[3d,4] in 50% Me_2SO–50% water at 20 °C.

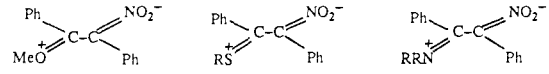


4 5 6 (2)

Upon mixing **4** with RS^- a rapid reaction is observed, which leads to a species whose absorption spectrum is distinctly different from that of **4** and **6** (Figure 1). The similarity of this spectrum to that of thiolate ion adducts of α-nitrostilbenes[5] and the fact that the intermediate slowly converts to **6** constitute strong evidence that the intermediate is **5**. The kinetics of intermediate formation and conversion to products were measured separately. Rates of reversible formation of **5** were determined in a stopped-flow apparatus by monitoring the loss of **4** at 340 nm. The observed pseudo-first-order rate constants ($[RS^-] \gg [\mathbf{4}]_0$) obey eq 3. Rate and equilibrium constants for four different thiolate ions are summarized in Table I.

$$k_{obsd} = k_1[RS^-] + k_{-1} \quad (3)$$

From plots of log k_1 and log k_{-1} vs log K_1 (not shown) one derives $\beta^n_{nuc} = 0.10 \pm 0.02$, $\beta^n_{lg} = -0.90 \pm 0.02$, and log $k_0 = 2.16$, with k_0 being the *intrinsic* rate constant defined as $k_1 = k_{-1}$ when $K_1 = 1$. This compares with $\beta^n_{nuc} = 0.19 \pm 0.03$, $\beta^n_{lg} = -0.81 \pm 0.10$, and log $k_0 = 3.43$ for the reaction of the same thiolate ions with α-nitrostilbene.[5] The reactions of **4** have K_1 values that are approximately 10^3-fold lower than the corresponding values for α-nitrostilbene,[5] probably due to a combination of resonance stabilization of β-methoxy-α-nitrostilbene (**4a**) and



4a 6a 7

steric crowding in **5**. The lower *intrinsic* rate constant for the reaction of **4** compared to the reaction of α-nitrostilbene may, at least in part, be attributed to loss of the resonance stabilization of **4** being ahead of C–S bond formation at the transition state.[6]

The conversion of **5** to **6** is very slow, which made it somewhat difficult to obtain good kinetics, presumably because of some oxidation of the thiolate ion. This latter could not be completely suppressed, despite standard precautionary measures. The most reproducible results were obtained with $RS^- = HOCH_2CH_2S^-$, keeping $[RS^-]$ close to 0.05 M and using the method of initial rates during the first 10% of the reaction. Repetitive HPLC analysis of the reaction mixture showed that **6** was the only product formed within this time period.[7,8] A $k_2 = 9.6 \times 10^{-6}$ s^{-1} was obtained.

Why is **5** the first intermediate reported to accumulate during a nucleophilic vinylic substitution? Three conditions are necessary for **2** or **5** to be observable. (1) The equilibrium of the first step must be favorable, i.e., $K_1[Nu^\nu] > (\gg) 1$. (2) The decay of the intermediate must be slower than its formation, i.e., $k_1[Nu^\nu] > (\gg) k_2$. (3) The absolute value of k_2 must be low enough to allow detection by suitable techniques, e.g., UV–vis in a conventional or stopped-flow spectrophotometer. In view of the fact that these conditions are so amply met in our system, it seems surprising that **2** has not been observed in other systems.

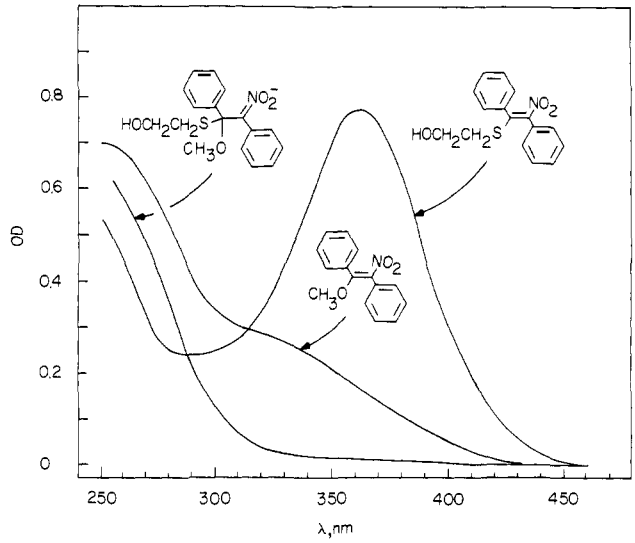


Figure 1. Absorption spectra of **4**, **5**, and **6** in 50% Me_2SO–50% water at 20 °C. $[\mathbf{4}] = [\mathbf{6}] = 8.33 \times 10^{-5}$ M. **5** was generated from 8.33×10^{-5} M **4** by adding 0.05 M $HOCH_2CH_2SH$ in a DABCO buffer at pH 9.0.

We can identify four factors that all conspire to make **5** easily detectable in our system: (1) the high nucleophilicity (high k_1) and high carbon basicity (high K_1) of thiolate ions;[9] (2) the strong stabilization of the negative charge by the nitro group[10] (high K_1, low k_2); (3) the low nucleofugality (leaving group reactivity) of methoxide ion[12] (low k_2); (4) the low intrinsic rate constants in nitronate ion forming/consuming reactions, especially in hydroxylic solvents[6] (low k_2). It appears that if one or more of these factors are absent, **2** is undetectable. For example, when LG in **4** is changed to I, Cl, or 4-MeC_6H_4O, substitution occurs without detection of the intermediate,[8] apparently because of the much higher nucleofugality of LG (higher k_2) and increased steric crowding in the intermediate (lower k_1, higher k_2). The same is true for the reaction of 4-$ClC_6H_4S^-$ with β-chloro- and β-iodo-α-nitrostilbene in ethanol and methanol.[9d] In the reaction of **4** with pyrrolidine and *n*-butylamine it is apparently the reduction in nucleophilicity[9] (lower k_1), coupled with an increase in k_2 induced by more crowding in the intermediate and a large resonance stabilization of the product (**7**), which leads to $k_1[Nu^\nu] \ll k_2$ and thus to undetectability of the intermediate.[8] In the substrates with different XY's such as $(CN)_2$, $(COOR)_2$, or (CN)-COOR for which kinetic data on nucleophilic vinylic substitution

(4) The reactant and product are shown as the E isomers although we have no definitive proof of this.

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(10) If the pK_a values of the carbon acids CH_2XY in water[12] are used as a measure of carbanion stabilization, the $Ph(NO_2)$ combination is one of the most effective ones, at least in hydroxylic solvents.

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are available,[1,2] carbanion stabilization is smaller[10] (lower K_1, higher k_2), and the intrinsic rate constants are higher[6,9c] (higher k_2).

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Hammett Analysis of Enzyme Action in Organic Solvents

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Among the means available in physical organic chemistry to probe transition-state structure, linear free energy correlations of structure and reactivity have been the most valuable.[1] This methodology, specifically Hammett analysis, has also been profitably used in mechanistic enzymology.[2] A major recent development in biochemistry is enzymatic catalysis in anhydrous organic solvents.[3] The ability of enzymes to function as catalysts in water-free media poses a challenging fundamental question of whether enzymatic reaction mechanisms in such media are the same as in aqueous solution. This issue is directly addressed in this study using Hammett analysis.

We selected a protease from *Bacillus licheniformis*[4] (subtilisin Carlsberg)[5] as a model for our investigation. This enzyme, whose physiological role is to hydrolyze water-soluble proteins in aqueous solutions,[5] is nevertheless catalytically active in a number of anhydrous organic solvents[6] (allowing for useful preparative transformations[7]); furthermore, substrate[8] and enantiomeric[9] specificities of subtilisin in organic media are radically distinct from those in water.[8,9] In the present work, we kinetically investigated subtilisin-catalyzed cleavage of para-substituted phenyl acetates (nitro-, acetyl-, chloro-, methyl-, and methoxy-) in water (hydrolysis) and in five anhydrous organic solvents (transesterification with 1-hexanol). Figure 1 depicts the dependencies

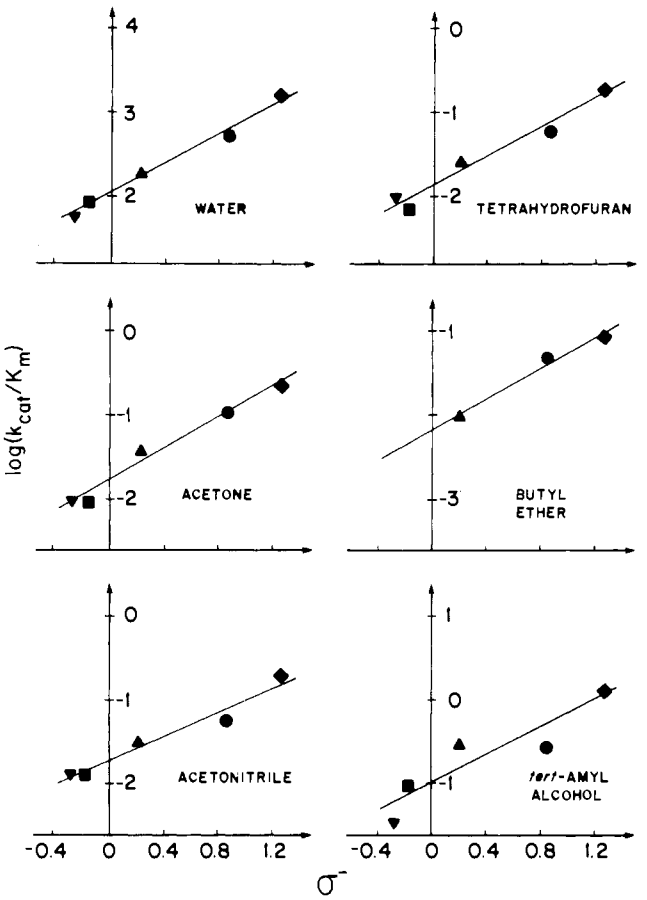

Figure 1. Hammett correlations for k_{cat}/K_m of subtilisin-catalyzed cleavage (hydrolysis in water and hexanolysis in organic solvents)[10] of para-substituted phenyl acetates. Symbols for the substituents are as follows: (♦), NO_2; (●), CH_3CO; (▲), Cl; (■), CH_3; and (▼), CH_3O. Conditions: (i) For enzymatic hydrolysis in water, substrate concentrations were varied from 0.2 to 1.3 mM, concentrations of the enzyme were in the range from 5.5 to 16.5 mg/L, pH 7.75 (20 mM phosphate buffer containing 2% acetonitrile), 30 °C; all reactions were followed spectrophotometrically as described in the literature.[17] (ii) For enzymatic transesterifications with 1-hexanol in anhydrous organic solvents, phenyl ester concentrations were varied from 10 to 100 mM, hexanol concentration was 1 M, and the concentration of the enzyme (lyophilized from the phosphate buffer, pH 7.75, as previously described[6]) was 1 mg/mL. All reactions were carried out at 30 °C with shaking at 300 rpm and were followed by capillary gas chromatography as described earlier.[6] Organic solvents were of analytical grade and were dried by shaking overnight with 4 Å molecular sieves prior to use. In the case of butyl ether as a solvent, enzymatic reactions with *p*-methyl- and *p*-methoxyphenyl acetates were too slow to measure accurately. The units of k_{cat}/K_m are $M^{-1}\cdot s^{-1}$.

of k_{cat}/K_m[10] on the substituent constant σ^- for both aqueous and nonaqueous reaction media. One can see that in each instance a satisfactory linear dependence is obtained (all correlation coefficients greater than 0.9), thereby allowing for the determination of the reaction constant ρ[11] (Table I).

Inspection of the subtilisin data in Table I reveals that in all solvents the ρ values are between 0.72 and 0.93, and the ρ value for the enzymatic reaction in water is near the middle of this range. The organic solvents employed in these experiments are both water-immiscible (butyl ether and *tert*-amyl alcohol) and

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