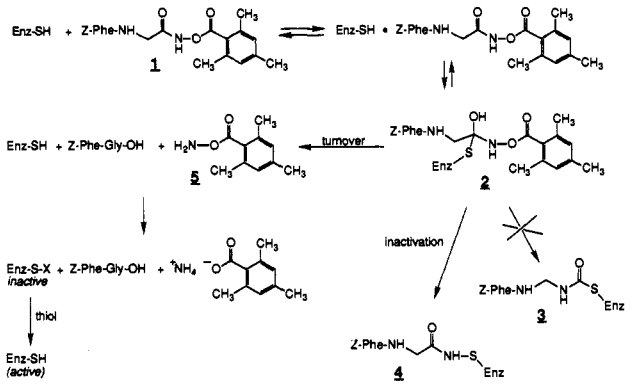
Scheme I. Proposed Scheme for the Mechanism of Inhibition of Papain-Type Cysteine Proteinases with Peptidyl *O*-Acylhydroxamates (e.g., 1)



of papain (in the absence of thiol) with 1 molar equiv of authentic **5**,[7] the formal hydrolysis product and potential "oxidant" (Scheme I), rapidly provided inactive papain, from which enzyme activity could again be mostly recovered by treatment with thiol.

To discriminate between the alternative adducts **3** and **4** proposed for the inactivation process[2] which occurs *in the presence of thiol*, papain was treated with labeled inhibitors **1a–c**. Using excess inhibitor **1a**,[8] ^{13}C NMR spectroscopy revealed a new, strong signal in the protein fraction for the ^{13}C-labeled carbonyl carbon at 182 ppm. Using **1b**, which has J_{C-C} = 53 Hz (acetone-d_6), an enzyme–inhibitor adduct was obtained that exhibited a carbonyl signal centered at 182 ppm with J_{C-C} = 50 Hz, thereby ruling out the thiolcarbamate structure **3**.[9] Finally, experiments were conducted with ^{13}C,^{15}N-labeled **1c**. In **1c**, J_{C-N} = 9 Hz was observed; in the enzyme–inhibitor adduct from **1c**, the line width of the ^{13}C carbonyl signal rendered measurement of a J_{C-N} coupling constant of ca. 9 Hz as plausible, but not conclusive. Unequivocal evidence for incorporation of the hydroxamate nitrogen atom in the adduct was obtained by measurement of a broad ^{15}N NMR signal at −274 ppm (relative to nitromethane at 0 ppm). This observed ^{15}N NMR chemical shift[10] agrees well with that measured for a model sulfenamide[11] ($CH_3CONHSCH_2CH_2CH_3$; ^{15}N NMR: −284 ppm (neat sample)).

In summary, we have identified two modes of inhibition of cysteine proteinases by peptidyl *O*-acylhydroxamates (Scheme I). *In the absence of a reducing thiol*, treatment of papain with a single molar equivalent of inhibitor **1** affords an inactive, apparently oxidized form of papain; enzymatic activity can be recovered by treatment with reducing thiol. Our evidence suggests that the expected turnover product *O*-mesitoylhydroxylamine **5** is the oxidizing agent in this process. *In the presence of thiol*, the turnover (hydrolysis) of inhibitor is followed by oxidation/reduction to give a complete catalytic cycle; this process competes with the inactivation of papain with a partition ratio of ca. 12. ^{13}C and ^{15}N NMR studies of the covalent inactivation adduct have provided evidence that rules out a thiolcarbamate structure **3**, while providing strong support for the novel sulfenamide adduct structure **4**. Further mechanistic studies are in progress.

Acknowledgment. We are grateful to Leslie J. Copp for her contributions to the enzymology and helpful discussions and to the Biotechnology Research Institute, Montreal, Quebec, Canada, and Bruker (Canada) for use of NMR instrumentation.

Supplementary Material Available: Information for syntheses of **1a–c** and NMR experiments and ^{13}C NMR spectra of papain adducts (2 pages). Ordering information is given on any current masthead page.

(7) (a) Marmer, W. N.; Maerker, G. *J. Org. Chem.* **1972**, *37*, 3520–3523. (c) Carpino, L. A. *J. Am. Chem. Soc.* **1960**, *82*, 3133–3135.

(8) Conditions: 15 equiv of labeled inhibitor **1**, pH 7, 10 mM potassium phosphate, 5 mM 2-mercaptoethanol. Small molecules (MW <10000) were removed from the inactivated enzyme by Sepharose chromatography.

(9) J_{C-C} values for CH_3NHCO are <5 Hz; see p 135 in the following: Wray, V.; Hansen, P. E. *Annu. Rep. NMR Spectrosc.* **1981**, *11A*, 99–181.

(10) Witanowski, M.; Stefaniak, L.; Webb, G. A. *Annu. Rep. NMR Spectrosc.* **1981**, *11B*, 1–486.

(11) (a) Harpp, D. N.; Mullins, D. F.; Steliou, K.; Triassi, I. *J. Org. Chem.* **1979**, *44*, 4196–4197. (b) Kise, H.; Whitfield, G. F.; Swern, D. *J. Org. Chem.* **1972**, *37*, 1125–1128.

Intramolecular Reaction Rate Is Not Determined Exclusively by the Distance Separating Reaction Centers. The Kinetic Consequences of Modulated Ground State Strain on Dyotropic Hydrogen Migration in Systems of Very Similar Geometric Disposition

Leo A. Paquette* and George A. O'Doherty[1]

Evans Chemical Laboratories, The Ohio State University Columbus, Ohio 43210

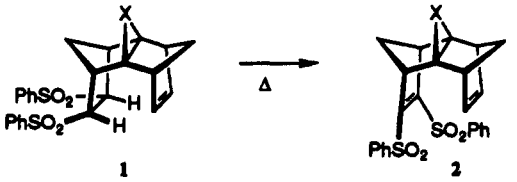
Robin D. Rogers[2]

Department of Chemistry, Northern Illinois University DeKalb, Illinois 60115

Received June 21, 1991

The ability of enzymes to alter state changes within suitable substrates with resultant dramatic acceleration of chemical reactivity has promoted extensive inquiry into the origin of this kinetic phenomenon.[3] The most recent of several controversies to surround this subject finds Menger and Houk as proponents of radically differing viewpoints. The "spatiotemporal hypothesis" holds that rates of enzymatic and intramolecular processes correlate strictly with the time that the reacting centers reside within a critical geometric disposition;[4] "sustained proximity at close distances...generates enzyme-like rates".[4c] The contrary argument holds that "the real determinant of reactivity is the energy required to distort the reacting functional groups into the geometry of the rate-determining transition state."[5,6]

There exists no question that distance can be an important determinant of rate. Relevantly, the first-order isomerizations of **1** to **2** have been shown to correlate very well with the intracavity distance (as determined crystallographically in every instance).[3]



1 2

A 0.1-Å change in the gap dimension separating the α-sulfonyl hydrogens from the sp^2-hybridized carbons in **1** translates into a rate spread of 10^4! Despite the excellent correlation observed, it is important and relevant to question whether proximity by itself suffices as a complete explanation. The structural relationships

(1) BP America Fellow, 1991; National Need Fellow, 1989–1990; University Fellow, 1988–1989.

(2) Author to whom inquiries relative to the X-ray crystallographic studies should be addressed.

(3) For a leading reference, see: Paquette, L. A.; Kesselmayer, M. A.; Rogers, R. D. *J. Am. Chem. Soc.* **1990**, *112*, 284.

(4) (a) Menger, F. M.; Venkataram, U. V. *J. Am. Chem. Soc.* **1985**, *107*, 4706. (b) Menger, F. M. *Acc. Chem. Res.* **1985**, *18*, 128. (c) Menger, F. M. *Adv. Mol. Model.* **1988**, *1*, 189. (d) Sherrod, M. J.; Menger, F. M. *J. Am. Chem. Soc.* **1989**, *111*, 2611. (e) Menger, F. M.; Sherrod, M. J. *J. Am. Chem. Soc.* **1990**, *112*, 8071. (f) Sherrod, M. J.; Menger, F. M. *Tetrahedron Lett.* **1990**, *31*, 459.

(5) (a) Dorigo, A. E.; Houk, K. N. *J. Am. Chem. Soc.* **1987**, *109*, 3698. (b) Dorigo, A. E.; Houk, K. N. *Adv. Mol. Model.* **1988**, *1*, 135.

(6) More recently, Sherrod and Menger have recognized that carbon skeleton distortion in a reactant ground state comprises most of the activation energy of a hydride transfer and that "only after a critical distance is reached does actual chemical reaction begin".[4f]

0002-7863/91/1513-7761$02.50/0 © 1991 American Chemical Society

Table I. Average Intragap Distances,[a] Absolute and Relative Rate Data, and Equilibrium, Constants for 3–8 (160 °C)

compd	av distance, Å	k_{for}, s^{-1}	K_{eq}	E_a, kcal/mol	k_{rel} (160 °C)
3a[b]	2.41	4.25×10^{-4}	1.8		24
3b	2.41	2.40×10^{-3}	20	26.2	130
4a	2.32	8.03×10^{-3}	2.8	27.2	450
4b	2.25	1.19×10^{-1}	56	24.0	6700
4c	2.35	1.09×10^{-2}	36.5	27.2	610
5a[b]	2.53	1.80×10^{-5}	5.8		1
5b	2.34	2.56×10^{-4}	50	29.8	14
5c	2.45	2.19×10^{-4}	24	31.3	12
6	2.44	2.28×10^{-3}	60	25.2	127
8	2.30	2.20×10^{-1}	>200	24.4	12000

[a] Nonbonded C(1)-H C(7) and C(2)-H C(6) distances have been averaged. See ref 3 for numbering. [b] Data taken from ref 3.

existent in **1**, although capable of an impressive rate profile, might not be sufficiently distinctive in other ways that would be reflected in the energetics of hydrogen dyotropy.

Presently, success has been realized in preparing several additional *syn*-sesquinorbornene disulfones having phenylsulfonyl groups more sterically compressed than those found in **1**. *Since the added ground-state energy in these compounds is not accommodated by heightened intragap compression, the associated transition state energy barriers are significantly lowered, with resultant kinetic acceleration relative to the norm.*

Diels–Alder cycloaddition[7] of (*Z*)-1,2-bis(phenylsulfonyl)-ethylene to the appropriately substituted tricyclo[5.2.1.$0^{2,6}$]-deca-2,5,8-triene[8] provided convenient access to the desired *syn*-sesquinorbornadienes. Subsequently, the central double bond in these adducts was regioselectively reduced (HN=NH), cyclopropanated (CH_2N_2; *hν*), or epoxidized (MCPBA). All of the end products proved to be colorless solids, the crystalline quality of which allowed for detailed structural analysis by X-ray methods.

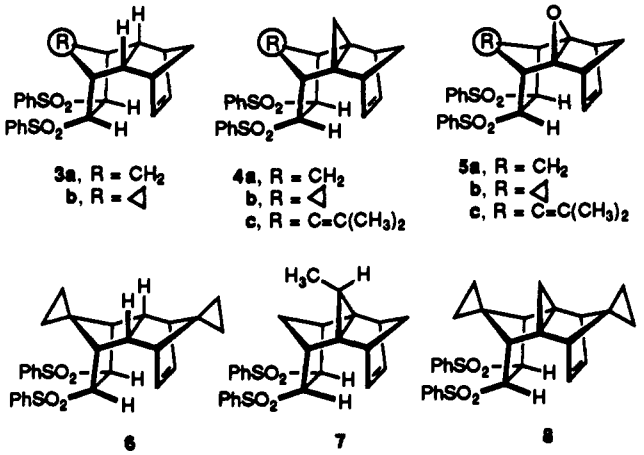


Table I summarizes the combined results of the crystallographic and kinetic measurements. The absolute rate constants, determined in C_6D_5Br solution with monitoring by 300-MHz ^1H NMR, were necessarily obtained over a wide temperature range (80–160 °C) because of the appreciably different reactivities. Compilation of the k_{rel} values was made possible by determining k_{for} at two temperatures for all new disulfones with extrapolation to 160 °C as necessary. In all cases, the exclusive product was the dyotropisomer corresponding to **2**.

Several important trends are reflected in the kinetic data. The incorporation of a proximal apical cyclopropane ring as in **3b**, while exerting no measurable change in compression level within the intracavity space relative to **3a** (r = 2.41 Å), so affects the intramolecular crowding experienced by the exo-oriented $PhSO_2$ groups that **3b** isomerizes more than 6 times faster than its "parent" at 160 °C. The presence of a second apical cyclopropane subunit as in **6** induces little further alteration in r (now 2.44 Å); nonetheless, **6** is similarly hyperreactive (by a factor of 5 at 160 °C) relative to **3a**. As seen in Figure 1, the data points for this triad of diimide reduction products generate a line having a slope inverse in sign relative to that defined by the cyclopropane and epoxide series.

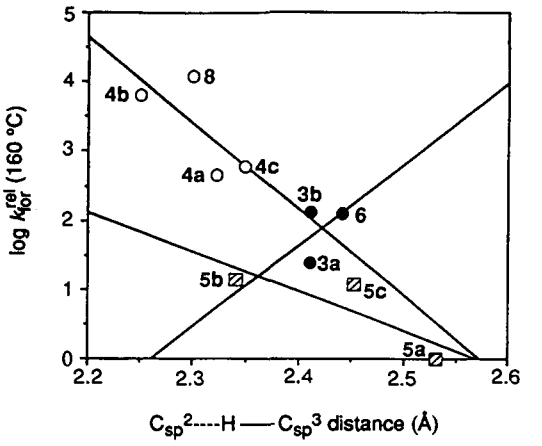


Figure 1. Plot of log k_{for}^{rel} (160 °C) versus the average $PhSO_2C$-H---sp^2-C distance.

An entirely similar kinetic relationship is exhibited by the subset comprising **4a**, **4c**, and **8**. The presence of the isopropylidene substituent in **4c** has little added impact on the intracavity gap compared to **4a** (2.35 vs 2.32 Å), but is 1.4-fold more reactive. As before, it is most reasonable to attribute this enhanced rate to newly introduced nonbonded interactions in the ground state of **4c** that are released while progressing to product. A comparison of the rate behavior of **4b** is equally revealing. Most notably the k_{rel} data for members of the cyclopropane series (viz., **4a–c** and **8**), when plotted in the conventional way, are widely scattered (Figure 1). Distance and rate are once again clearly not well correlated.

In contrast, the structural frameworks of epoxides **5a–c** appear more malleable. In these systems, an increase in steric congestion on the exo surface is mirrored systematically in a reduction of the magnitude of the $PhSO_2C$-H---sp^2-C distance. As a consequence, the associated kinetic differences give evidence of being controlled largely by proximity considerations. In agreement with this analysis, the slope of the line given by **5a–c** (−5.7, R = 0.85) compares very favorably to that originally recorded by Kesselmayer for **1** (−13, R = 1.0).[3]

The results presented above contraindicate the view that intramolecular dyotropic reactions are controlled exclusively by distance. Although proximity does play an important kinetic role, largely because the structural features of transition states are generally better simulated as reaction centers are brought incrementally closer, a more precise correlation must take proper cognizance of other key changes that perforce operate as rearrangement proceeds.[4f] These include, but are not necessarily limited to, those differences in the energy required to stretch bonds, variations in the extent of strain release, changes in freedom of motion, dissimilarities in developing π-electronic effects[9] that can gain importance in determining the magnitude of the energy barrier, and possible changes in reaction thermodynamics.[10,11]

(7) Paquette, L. A.; Künzer, H.; Green, K. E.; DeLucchi, O.; Licini, G.; Pasquato, L.; Valle, G. *J. Am. Chem. Soc.* **1986**, *108*, 3453.

(8) For the methods used, see: Paquette, L. A.; Kravetz, T. M.; Böhm, M. C.; Gleiter, R. *J. Org. Chem.* **1983**, *48*, 1250. Paquette, L. A.; Charumilind, P.; Kravetz, T. M.; Böhm, M. C.; Gleiter, R. *J. Am. Chem. Soc.* **1983**, *105*, 3126. Complete synthetic, spectral, and analytical details will be provided in the full paper.

(9) Mackenzie, K., private communication.

(10) This point is already reflected in the different K_{eq} values cited in Table I.

(11) This work was made possible by a grant from the National Cancer Institute (CA-12115), for which we are most grateful.