Geminal-Dialkyl Substitution, Intramolecular Reactions, and Enzyme Efficiency

Felice C. Lightstone[1] and Thomas C. Bruice*

Department of Chemistry
University of California at Santa Barbara
Santa Barbara, California 93106

Almost 75 years ago, Beesley, Thorpe, and Ingold[2] found that *gem*-dialkyl substitution on a chain connecting reacting functional groups was particularly effective in enhancing ring closure and retarding ring-opening reactions. The *gem*-dialkyl effect was proposed to arise from a decrease in the bond angle Φ brought about by repulsion between the two *gem*-substituents (Chart 1)—Thorpe−Ingold effect. Some 40 years later, Schleyer[3]

Chart 1

R′ ⁄⁄⁄ ⟩ Φ
R ⟋

concluded that the change in Φ was too small to afford an explanation of the large effects on reaction rates that had been observed on *gem*-substitution (e.g., 10^4−10^5-fold in ring closure[4]). At that time, Bruice and Pandit published studies on the influence of alkyl and *gem*-dialkyl substitution on the rate constant for intramolecular carboxylate displacement of *p*-bromophenol from mono-*p*-bromophenyl esters of glutaric acid.[5,6] These studies were extended[7a] to include hydrolysis of cyclic carboxylic acid anhydrides[7b] and pK_{a1} and pK_{a2} values of the dicarboxylic acids.[7c]

Bruice and Pandit[5] attributed the kinetic effect of *gem*-substitution on ring closure to a ground state phenomenon by a "decrease in the population of kinetically unprofitable extended rotamers and an increase in the population of rotamers which can undergo ring closure". For systems where geminal substitution increases rate constants for ring closure, the energetically favored ground state conformations were proposed to be extended. For those systems whose rates are rather insensitive to geminal substitution, the favored ground states were reasoned to be more or less closed.[8] Thus, it was suggested that mono- and *gem*-substitution increases the probability for formation of rotamers with the two ends arranged properly for the ring closing.[9] Now, some 35 years after the formulation of the reactive rotamer proposal, we report (i) a means of calculating the probability of formation of a given rotamer and (ii) the use

Chart 2
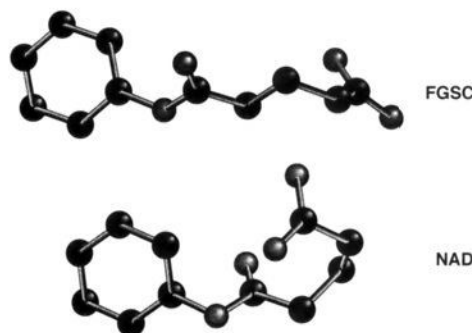
of this procedure in correlating the rate constants for intramolecular carboxylate displacement of *p*-bromophenolate from mono-*p*-bromophenyl esters of dicarboxylic acids to provide cyclic anhydrides. This accomplishment is important. Although most present arguments are in accord with the early concepts of Bruice and Pandit,[13] there are those who strongly disagree.[14]

With the exception of the *p*-bromophenyl ester of 3,6-endoxo-Δ^4-tetrahydrophthalate (**VI**), a search for all possible conformations was performed on each monoester (**I**−**VI**) using Saunders's stochastic search routine[15] in conjunction with MM3(92).[16] Through the use of SYBYL (v. 6.0.3),[17] the initial conformations were written to initiate the stochastic searches, and resulting conformations read into a database. Following the creation of $\geq 10\,000$ rotamer structures, duplicates and conformations with imaginary frequencies were eliminated. Statistical thermodynamic analysis from MM3 full-matrix minimization provided the final energy of each conformation, E_i, including the steric energy and electrostatic energy. For the *p*-bromophenyl ester of 3,6-endoxo-Δ^4-tetrahydrophthalate (**VI**), the MM3 dihedral driver was used to generate the unique conformations. The probability of formation of each local minimum conformer was calculated using a Boltzmann distribution (eq 1), where P_i is

$$P_i = \frac{e^{-\beta E_i}}{\displaystyle\sum_j^N e^{-\beta E_j}} \tag{1}$$

the probability of a given conformation i, E_i is the final energy of conformation i, N is the total number of conformations found, and β is $1/(kT)$, given that k is the Boltzmann constant and T is the temperature. Examination of eq 1 shows that the higher energy conformations will have the lowest probabilities. All minimizations and probabilities were calculated at $T = 298.16$ K and with a dielectric constant of 1.5.

The rotamer of lowest energy will be referred to as the favored ground state conformation (FGSC, Chart 2). Interestingly, the FGSC for glutarate was found to be an extended rotamer, while the FGSC found for the geminal substituted compounds were not extended to the same degree. This is in agreement with early suggestions of Bruice, Pandit, and Benkovic.[5,8]

The rotamer which meets the set criteria for preattack is called the near-attack conformation (NAC, Chart 2). In a separate experiment, AM1 calculations were employed to determine the distance at which van der Waals interactions begin within 3.2

(1) Contribution in partial satisfaction of the Ph.D. degree in chemistry.
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(9) Buttressing of a *gem*-dimethyl substituent can, in extreme cases, bring about conformations where there is overlap of van der Waals surfaces of reacting atoms (see refs 10 and 11). This can bring about increases in rate constants for ring closure of $>10^{11}$ (see ref 12).
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(16) Allinger, N. L.; Zhu, Z.-Q. S.; Chen, K. *J. Am. Chem. Soc.* **1992,** *114,* 6120 and references therein. Dr. Peter Fox kindly provided additional parameters assembled by N. L. Allinger, J. P. Bowen, and P. Fox.
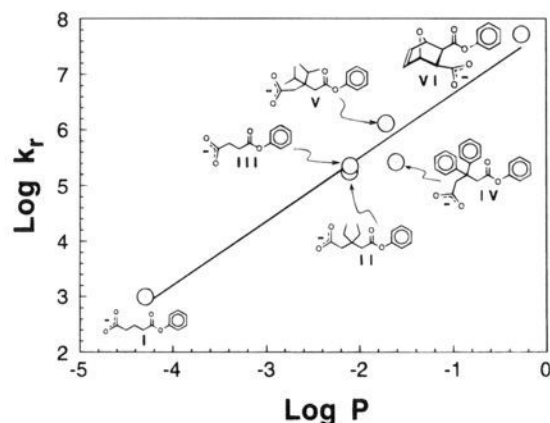(17) A software product of Tripos Associates, Inc. of St. Louis, MO.

Figure 1. Plot of the log of the relative rate constants (k_r) for anhydride formation from the mono-*p*-bromophenyl esters vs the log of the probability (P) for each monophenyl ester **I**–**VI** being in a near attack conformation. (For simplicity in determining P, computations of the distribution and stabilities of rotomers were carried out with monophenyl esters, corresponding to the mono-*p*-bromophenyl esters used in the kinetic studies.)

Chart 3
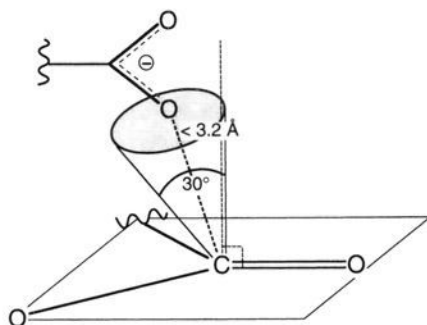
Å. The NAC had to meet two criteria: (i) the distance of approach of the nucleophilic oxygen to the carbonyl carbon had to be ≤ 3.2 Å and (ii) the approach of the nucleophile had to be within a cone of 30° with the axis being 15° off of the normal to the carbonyl plane (Chart 3).[18] A program was written to determine whether each unique local minimum conformation meets the NAC criteria. The overall probability of NAC formation (P) was taken as the sum of the probabilities of all individual NACs.

In Figure 1, the log of the relative rate constant for the intramolecular displacement at the ester carbonyl to provide anhydride (eq 2) is plotted against the log of the probability of
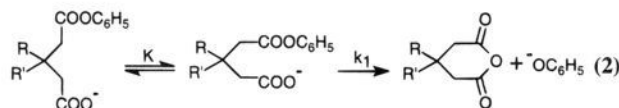
formation of the near attack conformation. Inspection of Figure 1 shows that a linear free energy relationship exists between $\Delta G^{\ddagger}$ and log P (eq 3) with slope $\cong 1$.

$$\log k_r = 1.15 \log P + 7.80 \quad (3)$$

For the moment, we will ignore the probality of NAC formation (the calculation of P involves consideration of all conformations) and compare (Table 1) the changes in standard free energy (ΔG°), enthalpy (ΔH°), and entropy (ΔS°) in going from the *energetically most stable* FGSC to the *energetically most stable* NAC for esters **I**–**VI**. Contrary to a common

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Table 1. Thermodynamic Parameters [MM3(92), $\epsilon = 1.5$] for NAC Formation with Monophenyl Esters

	ΔG° (kcal/mol)	ΔH° (kcal/mol)	ΔS° (eu)		ΔG° (kcal/mol)	ΔH° (kcal/mol)	ΔS° (eu)
I	4.92	4.66	−0.88	**IV**	−1.10	1.23	7.80
II	2.11	2.20	0.32	**V**	1.17	2.32	3.88
III	0.75	2.33	5.29	**VI**	0.11	0.09	−0.07

perception that the formation of a given conformation is entropy related, one clearly sees from Table 1 that there is no correlation between ΔS° and log k_r. However, there is a strong linear correlation between log k_r and ΔH° ($R = 0.93$).[19] In the class of intramolecular reactions, enthalpic factors control how easy it is to achieve the NAC.[21] The effect of geminal substitution on log k_r has been shown to be solely associated with changes in $\Delta H^{\ddagger}$, in $T\Delta S^{\ddagger}$, or in both.[13,22,23]

Biochemical Implications. In 1960, Bruice and Pandit[6] recognized that when P approaches unity (as in the case with **VI**), an ester effectively exists exclusively in the NAC with nucleophile and electrophile at ≤ 3.2 Å. They described the *propinquity effect* in these terms: "These experimental results point to the tremendous enhancement of rate that an enzyme could achieve by fixing the reactive species in a steric conformation closely resembling the transition state for the reaction," a concept indistinguishable from what Menger[24] was 25 years later to call the "spatiotemporal effect". In the present system, when $P = 1.0$, the relative rate constant (or EM value) equals $\sim 10^8$ M. To obtain rate constants much greater than that when $P = 1.0$ requires extreme steric compression with orbital overlap,[10] as seen in the trialkyl lock.[12]

In contrast, Page and Jencks[14] proposed that entropic contributions, from freezing translational and rotational motions and low-amplitude vibrations in the transition state, are the sole sources of rate acceleration in enzymatic and intramolecular reactions and for the chelate effect;[25] they rejected the role of rotomer distribution as "a factor of prime importance in intramolecular and enzymatic rate accelerations". They reasoned that the low energy barrier associated with free internal rotation around a C–C bond is ~ 4.5 eu and suggested that the freezing out of an internal rotation could produce a rate acceleration of only 5-fold. This oversimplification implies that all conformations have equal energetic stability. In fact, as Table 1 shows, the thermodynamic situation is complex, and the enthalpic contributions come far closer than the entropic contributions to describing the effect of conformational restrictions in the ground state. Thus, while the contributions of translational and rotational entropy to enzymatic accelerations of bimolecular reactions may be of some importance,[14] it is now apparent that the straightforward propinquity effect is a more reliable guide to internal rotational contributions than are simple entropy estimates.

(19) If the data points for the *gem*-diphenyl substituted ester (**IV**) are ignored, the correlation of ΔH° with log k_r is greatly improved ($R = 0.98$). Because of the crowding of *gem*-diphenyl substituents, the Ph–C–Ph bonds should be lengthened compared to the CH–Ph of a mono substitution (ref 20). This has not been well accounted for in these calculations, such that the calculated probability for the near-attack conformation is too large.
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