An Indication of the Magnitude of Orientation Factors in Esterification

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Abstract: The role of orientation in acid-catalyzed esterification reactions is evaluated by comparing intermolecular velocities and by correcting for other factors which affect reaction rates. After correction for proximity and torsional strain, factors of 10^4 are identified with the rate enhancement of some guided intramolecular reactions relative to their random bimolecular counterparts. Factors such as solvation, compression, and inductive effects were eliminated as sources of accelerations and ring strain corrections would, if anything, increase the ratio in the examples chosen. Data from equilibrium studies, nmr, infrared, and basic hydrolyses are presented. It is proposed that the optimization of orientation during reaction may be a key feature of enzyme action and that orientational constraints are one source for the widely varying rates of intramolecular cyclizations.

Enzymes are nature's most specific and powerful catalysts and no man-made catalysts have been capable of duplicating the great catalytic efficiency exhibited by enzymes under mild physiological conditions. The source of this exceptional catalytic power is of interest to biochemists and chemists alike because of its obvious importance to catalytic processes in general. Although the detailed mechanism is still not known for any enzyme, there are reasonable physical organic model systems for most enzyme-catalyzed reactions (*cf.* ref 1–5). However, a quantitative comparison between the velocity of these nonenzymatic analogs and the turnover numbers for enzyme-catalyzed reactions reveals rate differences as high as 10^{11} even after an attempt is made to correct for all the known catalytic features contributing to the activity of a specific enzyme.[3] Numerous explanations including strain,[6] multifunctional catalysis,[7] selective binding of transition states by enzymes,[8,9] electrostatic catalysis,[10] proximity,[11] and orientation[11] have all been considered as possible sources for the special catalytic power of enzymes. These various types of catalysis may all contribute to enzyme catalysis in some cases but quantitative evaluation of these catalytic modes and evidence for their actual involvement in enzyme catalysis has been elusive.

One property which most profoundly distinguishes enzymes from other catalysts is their ability to bind their substrates in close proximity to each other and to the catalytic groups on the enzyme. This enables the enzyme to accelerate a reaction by the juxtaposition of the reacting atoms, the proximity effect, and by their orientation. The question that arises is not whether these factors contribute to enzyme action but how much. However, in order to effect such an analysis the specific phenomena must be clearly distinguished and this is illustrated in Figure 1.

In Figure 1A, two molecules A and B are shown which might be substrates such as ATP and glucose. The dotted areas represent, respectively, the P atom undergoing attack in a kinase reaction and the O atom which acts as a nucleophile. The pie-shaped wedges represent the fraction of the solid surface over which reaction can occur. In this representation 1A represents the molecules in contact but the reactive atoms are neither juxtaposed nor oriented. In Figure 1B the reactive atoms are juxtaposed but not oriented. In Figure 1C they are both juxtaposed and oriented. In Figure 2 are shown two illustrations of the way in which an enzyme or an intramolecular compound can steer two reactants so that their reactivity is optimized.

The factor contributed by an enzyme which produces the juxtaposition in Figure 1B is called the proximity factor. If the reacting atoms had no orientational discrimination, all such juxtapositions would be reactive. If the electron orbital structure is not spherically symmetrical, however, reaction will occur more rapidly in certain directions. This dependence on orientation is shown schematically by the pie-shaped wedges. The pie-shaped wedges cannot be considered literally as a precise mechanical model any more than solid balls can represent real atoms. For one thing the orbital clouds do not end abruptly and p orbitals will extend in more than one direction. However, this schematic device is used to emphasize that the orientation factor which depends on entropy, bond bending, nonbonded interaction, and so forth[12,13] arises from an orbital structure which has a directional preference and that the rate enhancement resulting from this directional preference can be schematically represented as though it were the fraction of a solid surface over which reaction can occur.

The evidence that p, d, and hybrid orbitals have an angular component in their wave functions has been known for many years and is the contribution of many

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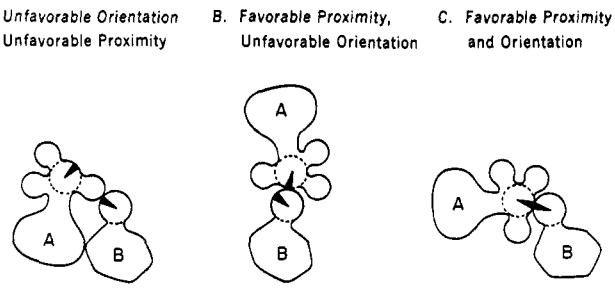

Figure 1. Illustration of proximity and orientation effects in a bimolecular reaction. (A) The molecules are in contact but no reaction occurs because reactive atoms are not juxtaposed. (B) The molecules are in appropriate proximity since the reactive atoms are juxtaposed. They will not react or will react very slowly, however, because the orbital alignments are unfavorable. (C) The molecules have the reactive atoms juxtaposed and the orbital orientations optimal.

individuals.[14-17] The question treated in the present study is the importance of this factor in chemical reactivity. Although calculations can be made on possible limits for bending vibrations, and vibration factors, the quantum mechanics of large systems are too complex to solve rigorously.[18] Accordingly, studies have been initiated to evaluate these factors experimentally. In the present study the quantitative estimations of orientation factors from intramolecular reaction rates are further elaborated and new experimental evidence for the hypothesis that "steering" reacting atoms can lead to large rate factors is presented.

Experimental Section

Synthetic Procedures. Infrared spectra were obtained with a Perkin-Elmer Model 257 grating infrared spectrophotometer using chloroform solutions or KBr pellets. Nmr spectra were recorded on a Varian Associates A-60 spectrometer using deuteriochloroform as a solvent and tetramethylsilane as an internal standard. In those cases in which higher resolution was required, nmr spectra were taken with a 220-MHz spectrometer made available through the kindness of M. Calvin of the Biodynamics Laboratory. Melting points wre uncorrected and were taken on a Thomas-Hoover capillary melting point apparatus. A Gilford recording spectrophotometer Model 2000 equipped with an insulated cell compartment which could be thermostated ±0.1° by means of a K-2/R Lauda/Brinkmann circulator was used to monitor the progress of reactions spectrophotometrically. A Radiometer pH-stat equipped with a thermostated sample vessel was used for kinetics and titrations. Gas chromatographic analyses were performed with an Aerograph Hy-Fi Model 328 using a 10% Carbowax column at 170°. Analyses were done by the microchemical analytical laboratory, University of California, Berkeley.

γ-Butyrolactone, γ-valerolactone, and phthalide were purchased from Aldrich Chemical Co. Phthalide was used without further purification and γ-butyrolactone and γ-valerolactone were distilled before use.

6-*endo*-Hydroxybicyclo[2.2.1]heptane-2-*endo*-carboxylic Acid Lactone. This lactone was synthesized by the method of Beckmann and Geiger.[19] The product was recrystallized from ethanol and vacuum sublimed: mp 157–158° (lit. mp 157–158°); ir ($CHCl_3$) 1776 (γ-lactone C=O); nmr ($CDCl_3$) δ 4.80 (t, 1, J_{6-1} = 4.7 Hz, C-6 hydrogen), 3.20 ppm (t, 1, J_{1-2} = 4.2 Hz, J_{1-6} = 4.7 Hz, C-1 hydrogen).

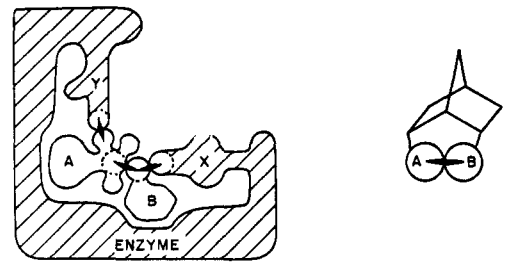

Figure 2. The constrained orientations in an enzymatic and an intramolecular reaction. The geometries of substrates and catalysts are constrained in the enzyme due to the three-dimensional structure of the protein and the specific binding of substrates. The catalytic groups are shown as side chains of amino acids and the substrates are bound to optimize orientation not only of the substrates relative to each other but also relative to the catalytic groups. Three different reactive pairs of optimized orientation are thus aligned in this case. In the intramolecular reaction, the reactive atoms A and B are aligned optimally because of the structure of the intramolecular ring system which selects certain conformations. Unfavorable alignments would decrease the rate of the reaction.

Anal. Calcd for $C_8H_{10}O_2$: C, 69.54; H, 7.30. Found: C, 69.47; H, 7.29.

2-*endo*-Hydroxymethylbicyclo[2.2.1]heptane-3-*endo*-carboxylic Acid Lactone. This compound was synthesized by the method of Vaughan, *et al.*, from norborn-5-ene-2,3-*endo*-dicarboxylic anhydride:[20] mp 148–149° (lit. mp 148–149°); ir ($CHCl_3$) 1761 (γ lactone C=O); nmr ($CDCl_3$) δ 4.25 ppm (ABX, 2, J_{AX} = 3.0 Hz, J_{BX} = 6.7 Hz, γ hydrogens).

Anal. Calcd for $C_9H_{12}O_2$: C, 70.78; H, 7.87. Found: C, 71.61; H, 7.95.

2-*endo*-Bicyclo[2.2.1]heptanecarboxylic Acid. 5-*endo*-Bicyclo[2.2.1]hept-5-enecarboxylic acid prepared by means of the iodolactonization method[21] was catalytically reduced (Adam's catalyst) with hydrogen in ethyl acetate: mp 65–66° (lit. mp 64–66°); ir ($CHCl_3$) 1705 (carboxylic acid C=O); nmr ($CDCl_3$) absence of alkene hydrogens.

Anal. Calcd for $C_8H_{12}O_2$: C, 68.53; H, 8.65. Found: C, 68.15; H, 8.68.

2-*endo*-Methylbicyclo[2.2.1]heptane-3-*endo*-carboxylic Acid. 2-*endo*-Thiobenzylmethylbicyclo[2.2.1]heptane-3-carboxylic acid (5 g) (prepared from benzyl mercaptan and 2-*endo*-hydroxymethylbicyclo[2.2.1]-heptane-3-*endo*-carboxylic acid lactone)[22] and 2 g of Raney Nickel were refluxed for 24 hr in 100 ml of ethanol. The solution was filtered and the solvent was removed under vacuum. The residue was dissolved in 10% bicarbonate, washed with ether, and acidified to pH 1, and the product was extracted into ether. The ether solution was dried over anhydrous calcium sulfate and the solvent was removed. After recrystallization from ethyl acetate, 1.5 g of product was obtained: mp 56–57°; ir ($CHCl_3$) 1705 cm^{-1} (carboxylic acid C=O); nmr ($CDCl_3$) δ 1.06 pm, (d, 3, J = 6 Hz, methyl hydrogens).

Anal. Calcd for $C_9H_{14}O_2$: C, 70.10; H, 9.15. Found: C, 70.01; H, 9.07.

Ethyl γ-Hydroxybutyrate. γ-Butyrolactone (10 g; 0.116 mol) was hydrolyzed overnight with 125 ml of 1 *N* NaOH. The aqueous solution was then extracted with ether to remove any unhydrolyzed lactone and the pH of this solution was adjusted to pH 5.5 Triethyloxonium fluoroborate[23] (22 g) dissolved in 50 ml of dry acetonitrile was added dropwise to the hydrolysate over a 1-hr period while maintaining the pH at 5.5 with the addition of 1 *N* NaOH. The aqueous solution was then extracted with ether, and the ether solution was dried over anhydrous calcium sulfate and evaporated. The crude product was vacuum distilled to give 5 g

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of ester: mp 50° (4 mm); ir ($CHCl_3$) 1725 (ester C=O), 3450 cm^{-1} (hydroxyl); nmr ($CHCl_3$) δ 3.30 (5, 1, $HOCH_2$), 1.25 (t, 3, J = 7 Hz, H_3CCH_2), 4.13 (q, 2, J = 7 Hz, CH_2CH_2), 3.65 ppm (t, 2, J = 6 Hz, γ hydrogens).

Anal. Calcd for $C_6H_{12}O_2$: C, 54.52; H, 9.16. Found: C, 54.99; H, 9.19.

Ethyl 2-*endo*-Hydroxymethylbicyclo[2.2.1]heptane-3-*endo*-carboxylate. 2-*endo*-Hydroxymethylbicyclo[2.2.1]heptane-3-*endo*-carboxylic acid lactone (16.9 g; 0.110 mol) was hydrolyzed overnight with 125 ml of 1 *N* NaOH. Unhydrolyzed lactone was removed by ether extraction of the aqueous solution and this solution was adjusted to pH 5.70. Triethyloxonium fluoroborate[23] (22 g; 0.110 mol) dissolved in 50 ml of dry acetonitrile was added dropwise to the hydrolysate over a 2-hr period while the pH was maintained at 5.70 by the addition of 1 *N* NaOH. As the reaction proceeded the product precipitated out of solution and after all the fluoroborate was added, the ester was extracted into ether. The ether solution was dried over anhydrous calcium sulfate and evaporated. The infrared spectrum of the crude product showed equally intense carbonyl bands at 1725 and 1760 cm^{-1} indicating that the ester was contaminated with the γ lactone. The ester was separated from the lactone by silica gel chromatography using benzene as a solvent: mp 135–137°; ir ($CHCl_3$) 1725 cm^{-1} (ester C=O); nmr ($CDCl_3$) δ 3.35 (s, 1, $HOCH_2$), 4.15 (q, 2, J = 6.0 Hz, CH_3CH_2OC), 3.80 (m, 2, $HOCH_2$), 1.25 ppm (t, 3, J = 6.0 Hz, CH_3CH_2O). The ^{13}C nmr spectrum of this ester (section VII of the results) was also consistent with the assigned structure.

Anal. Calcd for $C_{11}H_8O_3$: C, 66.64; H, 9.15. Found: C, 66.48; H, 8.90.

Ethyl 6-*endo*-Ethoxybicyclo[2.2.1]heptane-2-*endo*-carboxylate. A solution of 11.7 g (0.07 mol) of sodium 6-*endo*-hydroxybicyclo-[2.2.1]heptane-2-*endo*-carboxylate in 70 ml of distilled water was cooled to 0° and the pH was adjusted to 9.0. A solution of 24.7 g (0.13 mol) of triethyloxonium fluoroborate[23] in 13 ml of dry acetonitrile was slowly dripped in. The pH of the reaction solution was maintained at 8.5–10.0 by simultaneously adding 3.5 *N* sodium hydroxide. After all of the triethyloxonium fluoroborate had been added, the pH continued to decrease indicating that the reaction was not instantaneous at 0°. Base was therefore added dropwise to maintain the pH at 8.5–10.0 until the pH remained constant. The resulting aqueous solution was extracted with ether, and the ether layers were combined, dried with anhydrous magnesium sulfate, and evaporated to give 11 g of oily solid. Infrared analysis of the crude product showed that it consisted primarily of lactone (1760 cm^{-1}), but a small amount of ester absorption at 1710 cm^{-1} was also apparent. Chromatography on silica gel using 8:2 petroleum ether–chloroform gave a series of fractions the first of which were oils. The remainder were crystalline solids. Infrared analysis indicated that the first four yellow fractions were mixtures of lactone and ester; the remaining fractions were almost exclusively lactone. The first four fractions were recombined and carefully rechromatographed on silica gel using 8:2 petroleum ether–chloroform. The first six yellow fractions were free of lactone. They were combined, dissolved in ether, dried with anhydrous magnesium sulfate, and decolorized with Norit to yield 0.74 g of colorless oil: nmr ($CHCl_3$) δ 1.10 (t, H_3CCH_2), 1.25 (t, H_3CCH_2), 3.38 (q, CH_3CH_2OR), 4.10 ppm (q, obscured partially by C_6 proton multiplet, CH_3CH_2OCR). In addition, the ^{13}C nmr of this compound was consistent with the assigned structure.

The sodium salts of the γ-hydroxy acids were prepared from the γ-lactones by hydrolysis with stoichiometric amounts of aqueous sodium hydroxide followed by filtration and lyophilization. In all cases, ir spectra (KBr pellets) of the hydrolysis products from the lactones showed the characteristic carboxylate anion absorbance at ~1430 and ~1600 cm^{-1}.[24] Further support for the structures assigned to the lactones was given by the observation that treatment of these hydrolysis products with 1 *N* KCl resulted in the formation of the appropriate lactone carbonyl peaks in the infrared.

Kinetic Procedures. With the exception of *o*-hydroxymethylbenzoic acid, the rates of acid-catalyzed lactonizations were monitored spectrophotometrically by the change in optical density in the range 230–238 mμ. The lactonization of *o*-hydroxymethylbenzoic acid to phthalide was followed by 254 mμ.[25] At substrate concentrations of approximately 1 × 10^{-2} *M* there were optical density changes from 0.05 to 0.2 optical density unit during the course of the reaction. These reactions were also followed, whenever feasible, by the hydroxamate assay for lactones.[26] In addition, the lactonization of 6-*endo*-hydroxybicyclo[2.2.1]heptane-2-*endo*-carboxylic acid was followed at pH 4.5 using a Radiometer pH-stat to record the uptake of hydronium ions. In all cases, the rates determined by several different techniques were identical.

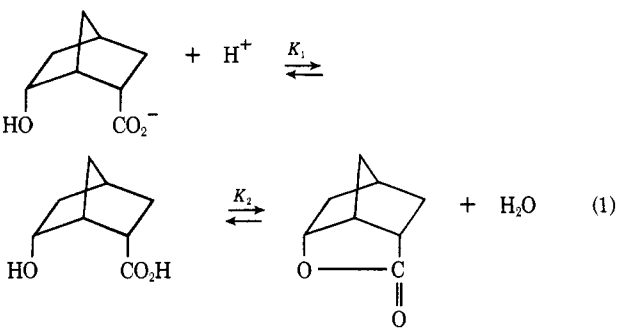
Kinetic runs were initiated by bringing a weighed amount of sodium salt of the γ-hydroxy acid to volume with the appropriate acid solution or buffer. The acidities ranged from 0.4 *N* HCl to 10^{-4} *N* in 0.5 *M* formate buffers. Reactions studied in the acidity range of pH 2 were done in 0.2 *M* Na_2SO_4–HCl buffers. All solutions were 20% (v/v) in ethanol with μ = 0.400. These reactions were studied at 25° unless otherwise specified.

First-order rate constants were determined from semilog plots of $(A_\infty - A_t)$ *vs.* time in the usual manner. The lactonizations were strictly first order in that they gave linear semilog plots through 2 or more half-lives and the observed first-order rate constants were independent of substrate concentration. All kinetics studied in buffers were done as a function of buffer concentration and rate constants at zero buffer concentrations were determined by extrapolation.

Basic hydrolyses of the lactones were followed spectrophotometrically at the same wavelengths used for each compound to study their lactonizations. The hydrolyses were carried out in 0.2 *M* phosphate buffers at pH 11–11.5 at 25°. In order to evaluate the buffer independent rate of hydrolyses these rates were also determined as a function of buffer concentration at constant pH and very small changes were observed for both phosphate and formate buffers. The reported rates are those extrapolated to zero buffer concentration.

Ionization Constants for the Carboxylic Acids. The ionization constants for the carboxylic acids were determined by potentiometric titration in 10% (v/v) ethanol, μ = 1.00 at 25°. Three determinations were made for each dissociation constant.

Equilibrium Constants for Lactone Formation. Initially, attempts were made to determine the equilibrium constants between the γ-hydroxy acids and their respective lactones by directly measuring the equilibrium concentration of lactones using the hydroxamate analysis for lactones.[26] In most cases, the equilibrium constants for lactone formation were much greater than 100. Because of the inaccuracy of the hydroxamate assay (±10%) and the large values of these equilibrium constants it was not possible to directly determine equilibrium constants for some crucial reactions of interest. It was decided that the equilibrium constant, K_2, could be determined by measuring the equilibrium concentration of the lactone at values of pH > pK_a of the acid. The ionization of the carboxylic acids can be used to pull the equilibrium in favor of the open-chain forms.



(1)

The following assay conditions were used to measure lactone concentration.

(1) The lactone solution (0.4 ml) (1 × 10^{-2} *M*) and 1 ml of basic hydroxylamine (1 *N* hydroxylamine in 1.75 *N* NaOH) were stirred for exactly 1 min. Zero time is taken when these two solutions have been added together.

(2) $FeCl_3$ (1.0 ml; 0.64 *M*) in 0.1 *N* HCl is added to the assay mixture followed by 0.3 ml of 4 *N* HCl.

(3) The solution is shaken vigorously and the optical density is read continuously at 540 mμ during the period t = 3 to t = 7 min. The absorbance at time zero is then determined by plotting the log of the optical density *vs.* time and extrapolating to time zero. Standard curves of lactone concentration *vs.* optical density at 540 mμ (time zero) were made for each lactone.

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(25) J. F. Burnett and C. F. Hauser, *J. Amer. Chem. Soc.*, **87**, 2214 (1965).

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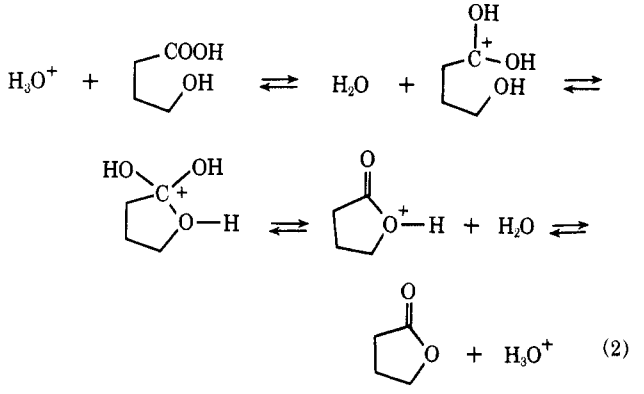
A weighed amount of lactone and sodium hydroxide at varying concentrations were brought to volume in 20% (v/v) aqueous ethanol. The solution was allowed to equilibrate until the pH stabilized and then the equilibrium concentration of the lactone was determined by the hydroxamate assay. The concentration of $RCOO^-$ and RCOOH were calculated from the pH of the solution, the pK_a of the acid, the total amount of lactone weighed into solution, and the equilibrium concentration of lactone. The equilibrium constant K_2 is defined as [equilibrium of lactone]/[equilibrium of RCOOH].

Results

Stoichiometry of Acid-Catalyzed Lactonizations. In order to confirm that only the expected lactonizations occurred under the conditions used for the kinetic studies, the following analysis was performed with each hydroxy acid. After the lactonization of the hydroxy acid was judged to be complete from its kinetic behavior (10 half-lives), the aqueous solution was extracted with chloroform and the product was identified from its melting point, infrared spectrum, and nmr spectrum. Under the conditions used for kinetic studies, all hydroxy acids were converted stoichiometrically to their repective lactones with no side products.

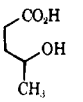
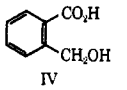
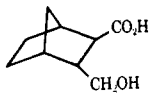
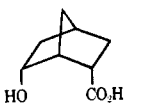
In general, acid-catalyzed esterifications and lactonizations of primary and secondary alcohols proceed with acyl–oxygen fission.[27-31] By analogy from the



(2)

studies done with γ-butyrolactone, the lactonizations reported in this study undoubtedly proceed by this mechanism although no direct evidence for this assumption is available. However, it is unlikely that any of these lactonizations proceed by carbonium ion mechanisms since the kinetic studies were done in weakly acidic solutions. Only the norbornyl alcohol would lead to exceptionally stable carbonium ions at the alkyl carbon[32] and it was studied at very low acidities. Furthermore, generation of carbonium ions in the 2-carboxynorbornane ring system results in the formation of a number of side products including the 2-*exo*-7-lactone.[19] These side products did not occur under the conditions used to study the lactonization of compound VI of Table I. Moreover, the thiolactonization of the γ-mercapto analog of the 2,6-norbornane compound has been studied and no hydrogen sulfide is produced by this reaction. The evidence indicates that acyl–oxygen fission occurs in all of these lactonizations.

Table I. Rates of Acid-Catalyzed Lactonizations and Basic Hydrolysis Rates

Substrate	Acidity range[a] over which lactonizations were studied, *M*	Acid-catalyzed lactonization, rate of M^{-1} min^{-1} [b]	Rate of basic hydrolysis, M^{-1} min^{-1} [c]
CH_3CH_2OH + CH_3CO_2H I	0.5–1.0	0.00109	6.6[d]
 II	0.1–0.4	0.086	123
 III	0.1–0.4	0.127	36
 IV	0.1–0.4	0.344	52
 V	0.05–0.2	7.23	13
 VI	1×10^{-4}–5×10^{-4}	1120	81

[a] HCl solutions were used for acidities in the range of 0.05–1.0 *N*. 0.5 *M* formate buffers were used in the acidity range 1×10^{-4}–5×10^{-4} *N*. [b] Second-order acid-catalyzed rate constants, 20% (v/v) ethanol, μ = 0.40, 25°. [c] Second-order base-catalyzed rate constants, 0.2 *M* phosphate buffers at pH 11–11.5 at 25°. [d] J. P. Kirsch and W. P. Jencks, *J. Amer. Chem. Soc.*, **86**, 839 (1964).

Acid-Catalyzed Rate Constants. The lactonization and esterification rates were acid catalyzed and studied over a range of acid concentrations as shown in Table I. It was established in each case that the rate was linearly proportional to the hydrogen ion concentration and the rates extrapolated to 1 *M* H^+ are recorded in Table I. Because salt effects have been observed with the acid-catalyzed hydrolysis of esters,[33] the rates were studied at identical ionic strengths (μ = 0.40) and solvent compositions (20% v/v ethanol).

The basic hydrolyses of the lactones were measured in a similar manner and the rates normalized to 1 *M* OH^- are also summarized in Table I.

It is readily seen that the acid-catalyzed esterification rates vary by a factor of 10^6 whereas the basic hydrolysis of the lactones vary by a factor of about 10. Large rate increases in intramolecular systems have been observed by others.[25,34-36] This series has some unique properties, however, in that the rings are all five-membered saturated rings which were selected

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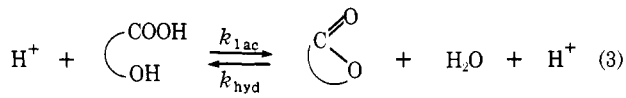
(29) S. G Cohen and A. Schneider, *J. Amer. Chem. Soc.*, **63**, 3382 (1941).
(30) F. A. Long and L. Friedman, *ibid.*, **72**, 3692 (1950).
(31) D. N. Kursanov and R. V. Kudryautsev, *Zh. Obshch. Khim.*, **26**, 1040 (1956).
(32) D. G. Sargent, *Quart. Rev., Chem. Soc.*, **20**, 301 (1966).
(33) R. A. Robinson, *Trans. Faraday Soc.*, **26**, 217 (1930).
(34) T. C. Bruice and U. K. Pandit, *Proc. Nat. Acad. Sci. U. S.*, **46** 402 (1960).
(35) S. Milstien and L. A. Cohen, *ibid.*, **67**, 1143 (1970).
(36) M. L. Bender, *Chem. Rev.*, **60**, 53 (1960).

to minimize inductive effects and ring strain. In order to do this, ring systems containing double bonds or hetero atoms were avoided in the rate comparisons. The rates of acid-catalyzed esterifications are quite insensitive to inductive effects as indicated by a $\rho = -0.2$ to $+0.5$ for the acidic hydrolyses of ethyl benzoates.[37] Furthermore, simple γ lactonizations proceed by the same mechanisms as esterifications so that there are no mechanistic ambiguities clouding the direct comparison between the intramolecular and intermolecular rates.

Equilibrium Constants. Although all the acid-catalyzed reactions are in principle reversible, in practice it is not easy to measure reverse rates for reactions which proceed to more than 99% completion in the forward direction. The rate constants for the back reactions were determined by measuring the equilibrium constant and dividing by the forward rate. By increasing the pH of the equilibration mixtures to higher values, the equilibrium was altered so that appreciable amounts of hydroxy acid were present in the ionized form.

The rate constant for a lactonization which is approaching equilibrium as given in eq 3 can be expressed in terms of eq 4.[38]



(3)

$$k_{lac} = k_{obsd}\left[\frac{[\text{lactone}]_{eq}}{[\text{lactone}]_{eq} + [\text{hydroxy acid}]_{eq}}\right] \quad (4)$$

γ-Hydroxybutyric acid and γ-hydroxyvaleric acid are converted to equilibrium mixtures which are 86 and 92% lactone and these equilibrium constants were determined by direct application of the hydroxamate assay.[26] The equilibrium constants are reported in Table II.

Table II. Equilibrium Constants for Lactone Formation and Rates of Acid-Catalyzed Hydrolyses

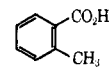
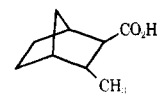
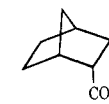
Substrate (as in Table I)	$k_{lacton}^{H^+}$,[a] M^{-1} min^{-1}	K_{eq}	$k_{hydrol}^{H^+} \times 10^3$, M^{-1} min^{-1}
I	1.09×10^{-3}	7×10^{-2}[b]	15.6
II	8.58×10^{-2}	6.15	14.0
III	1.27×10^{-1}	11.13	11.4
V	7.23	2,810	2.6
VI	1120	12,740	88.1

[a] Second-order acid-catalyzed rate constants, 20% (v/v) ethanol, μ = 0.40, 25°. [b] J. Gerstein and W. P. Jencks, *J. Amer. Chem. Soc.*, **86**, 4655 (1964); A. Kailan, *Z. Phys. Chem. (Leipzig)*, **101**, 63 (1922).

The equilibrium constants varied over six orders of magnitude and in general paralleled the acid-catalyzed lactonization rates. From the equilibrium constants and the lactonization rates the acid-catalyzed hydrolysis rates can be calculated and these are also shown in Table II. They vary over a range of 34 and are in general more like the basic hydrolysis rates in their invariance with the ring structures in this series of compounds.

Ionization Constants for the Carboxylic Acids. The ionization constants of the carboxylic acid were needed to calculate the equilibrium constants and also to serve as an indication of solvation effects in the various compounds. Transfer from protic to aprotic solvents causes considerable pK and rate changes in many reactions.[39-41] A difference in ground-state solvation would be expected to have an effect on the pK values of the respective acids and these values were ascertained. Titrations were carried out at the same ionic strength, temperature, and solvent composition in all cases. The results are shown in Table III, together with the pK values of the corresponding deoxy analogs.

Table III. pK_a Values of the γ-Hydroxy Acids and Their Deoxy Analogs

Acid	pK_a[a]	ΔpK_a
$CH_3(CH_2)_2COOH$	4.82	
II	4.72	−0.10
	3.91	
IV	3.94	+0.03
	5.30	
V	5.09	−0.21
	5.30	
VI	5.44	+0.12

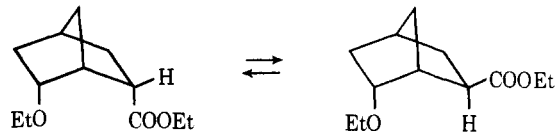
[a] Ionization constants for the carboxylic acids were determined by potentiometric titration in 10% (v/v) ethanol, μ = 1.00 at 25°.

It is readily seen that varying the structures of the hydroxy acids has a minor effect on the pK values of the carboxyl groups (a factor less than 10 for overall inductive and environmental effects) and that the pK changes of the deoxy acids compared to the hydroxy acids are uniformly small (0–0.21 pK unit). It can be assumed that either there is no major change in the solvation of the carboxyl group or that the pK measurements are insensitive to the changes which do occur.

Equilibration between 2-Exo and 2-Endo Isomers. The 2,6-norbornane hydroxy acid (VI of Table I) lactonizes one million times faster than the esterification of acetic acid and ethanol. Inasmuch as the hydroxyl and carbonyl groups are held rather tightly together in this compound, the possibility of rate accelerations by ground-state compression should be given serious consideration. When the Dreiding models of this derivative are made, the carbon atom of the carboxyl and the oxygen of the hydroxyl are 2.20 Å apart while the sum of their van der Waal's radii is 3.20 Å. This steric interaction could amount to many kilocalories of steric strain based on some theoretical van der Waal's

(37) R. W. Taft in "Steric Effects in Organic Chemistry," M. S. Newman, Ed., Wiley, New York, N. Y., 1956, p 556; R. W. Taft, *J. Amer. Chem. Soc.*, **74**, 3120 (1952); **75**, 4231 (1953).
(38) A. A. Frost and R. G. Pearson in "Kinetics and Mechanisms," Wiley, New York, N. Y., 1953, p 185.
(39) J. Miller and A. J. Parker, *J. Amer. Chem. Soc.*, **83**, 117 (1961).
(40) A. J. Parker, *Quart. Rev., Chem. Soc.*, **16**, 163 (1962).
(41) D. D. Roberts, *J. Org. Chem.*, **31**, 4037 (1966).

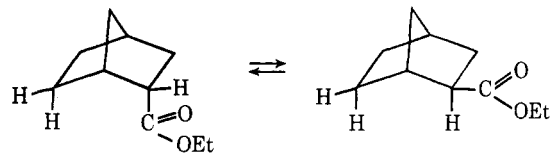
repulsion calculations.[32] To evaluate this possibility the equilibrium between the exo and endo isomers shown below was established since the equilibrium should favor the exo isomer if indeed there is much steric repulsion between the two endo substituents (eq 5, K_{eq} = 0.7). The purified endo ester was treated



(5)

with sodium ethoxide in ethanol until equilibrium was attained. Equilibration is achieved because of the ionizable α hydrogen atom and the equilibrium mixture was quantitated by gas-phase chromatography. At 25° the equilibrium constant was found to be 0.7.

The equilibrium constant for the equilibration of the exo,endo isomers of eq 5 indicates very little, if any, steric repulsion between endo-2,6 substituents. However, the repulsion might still be present if the normal exo to endo ratio highly favored the endo isomer. This latter possibility does not seem to be the case since the equilibrium constants for a number of exo,endo conversions have been obtained in the norbornane ring system and the exo isomers are usually favored some what over the endo isomers. For example, the equilibrium constant for the reaction shown in eq 6 is 3.0.[42]



(6)

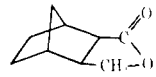
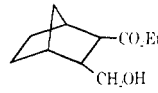
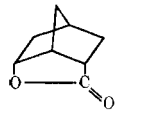
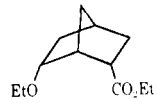
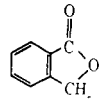
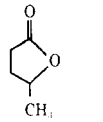
Similarly, 2-*exo*-norborneol is favored over its endo isomer by a factor of five- to sevenfold.[43,44]

The possibility that a favorable dipole–dipole interaction between the ethoxy group and the ester group compensates for steric compression between these two groups should be considered. Using a dielectric constant of 1, a distance of 2.2 Å between these two groups and partial charge of 0.1 charge unit gives a favorable dipole interaction energy of −2 kcal.[45] A dielectric constant of 10 would give a dipole interaction energy of only a few hundred calories. Thus the dipole interaction energy is not large and relief of ground-state strain cannot account for the high rate of lactonization of the *endo*-2,6-norbornane hydroxy acid.

Infrared and ^{13}C Nmr Data. The rates of the cyclization would be affected by any abnormal ring strain, steric hindrance, or other unusual properties of the reactants or products. To search for any such abnormalities, the infrared carbonyl stretching frequencies and the ^{13}C nmr spectroscopy of these molecules were examined. The results are shown in Table IV.

There is no evidence from the spectral properties of these compounds that either the lactone rings or their open-chained analogs are seriously strained. The infrared carbonyl stretching frequencies of the lactones vary only about 10 cm^{-1}. In general, the infrared carbonyl stretching frequencies are quite sensitive to

Table IV. ^{13}C Nmr Chemical Shifts for Lactones and Some of Their Open-Chained Analogs

Compd	Chemical[a] shift	Assignment	Ir carboxyl stretching frequency, cm^{-1}
	13.20	O=C	1773
	123.07	γ-C	
	164.53	α-C	
	170.13	β-C	
	16.83	O=C	1725
	129.87	γ-C	
	131.06	CH_3CH_2O	
	160.67	α-C	
	163.89	β-C	
	177.13	CH_3CH_2O	
	12.13	O=C	1761
	123.07	γ-C	
	145.00		
	149.87		
	151.40		
	151.40		
	166.35		
	170.32		
	16.73	O=C	1725
	130.00	γ-C	
	131.47	CH_3CH_2O	
	145.20		
	147.91		
	150.33		
	151.91		
	167.80		
	169.53		
	177.60	CH_3CH_2O	
	9.47	O=C	1776
	110.57	γ-C	
	145.25		
	152.60		
	153.57		
	153.88		
	155.17		
	157.31		
	16.15	O=C	1725
	110.53	γ-C	
	126.36	Ether, CH_3CH_2O	
	131.55	Ester, CH_3CH_2O	
	147.51		
	148.51		
	152.89		
	154.85		
	160.40		
	176.40	Ether, CH_3CH_2O	
	177.52	Ester, CH_3CH_2O	
			1778
			1773

[a] The ^{13}C shifts are in ppm from CS_2 and are considered accurate to ±0.3 ppm.

(42) A. C. Cope, *et al.*, *J. Amer. Chem. Soc.*, **81**, 799 (1959).
(43) C. H. Depuy and P. R. Story, *ibid.*, **82**, 627 (1960).
(44) C. F. Wilcox, M. Sexton, and M. F. Wilcox, *J. Org. Chem.*, **28**, 1079 (1963).
(45) C. P. Smyth in "Dielectric Behavior and Structure," McGraw-Hill, New York, N. Y., 1955, p 289.

ring strain.[46] For example, β-butyrolactone has a carbonyl peak at 1840 cm^{-1}, which is 70 cm^{-1} greater than that for γ lactones. ^{13}C nmr spectra of the lactones and some of their open-chained analogs were taken because it would be expected that any strain relief occurring upon cyclization would be expressed in chemical-shift changes. In the butyryl and 2,3-norbornyl ring systems the chemical shifts of the carbonyl carbon atom change 4–5 ppm upon cyclization whereas in the 2,6-norbornane system the carbonyl changes 7 ppm. The γ carbon of the butyryl and 2,3-norbornane ring system changes 7 ppm while in the 2,6-norbornane system the corresponding change is negligible. In general, there are not large shifts observable when comparing the open-chained esters with their corresponding lactones.

There is considerable evidence in the literature that ^{13}C chemical shifts are sensitive to steric interactions and this fact has been qualitatively demonstrated with norbornyl derivatives.[47] Although there has been some success in correlating ^{13}C chemical-shift changes with steric interactions, these calculations have not been reliable on a quantitative basis.[48] Therefore, it is not possible to assign energies to the small changes in chemical shifts observed with these cyclizations. The lack of large changes in the ^{13}C chemical shifts suggests, at least tentatively, that the large rate enhancements observed with the norbornyl compounds cannot be attributed to ground-state compression which is relieved upon cyclization.

Corrections for Torsional Strain. In some of the ring closures the cyclization results in partial eclipsing of vicinal hydrogens and this unfavorable torsional strain would have a tendency to decrease the rate. To correct for this factor the energy of these interactions were calculated using the Karplus nmr relationship[49] and the dependence of torsional energy on the dihedral angles[50] as shown in eq 7. These corrections have been widely

$$V(\theta) = {}^1/_2V_0(1 + \cos 3\theta) \qquad (7)$$

used.[51–54] Contrary to some criticisms of these corrections[55] the coupling constants can be determined for these systems. Here V_0 is the height of the potential barrier which is 1 kcal for a pair of eclipsed hydrogens. As a first approximation, it was assumed that the orientations of the hydrogens in the lactone were the same as the hydrogens in the cyclic transition state. This gave values of 2.5, 1.88, and 0.93 kcal for the torsional strain energies in compounds II, IV, and V, respectively. No torsional strain would occur in the bimolecular reaction and no change in the dihederal angles between vicinal hydrogens occurs in the 2,6 derivatives. When the energies are converted to rate factors, values of 64, 21.9, and 4.5 are obtained for compounds II, III, and V, respectively.

(46) L. J. Bellamy in "The Infrared Spectra of Complex Molecules," Wiley, New York, N. Y., 1958.
(47) J. B. Grutzner, M. Jautelat, J. B. Dence, R. A. Smith, and J. D. Roberts, *J. Amer. Chem. Soc.*, **92**, 7107 (1970).
(48) D. M. Grant and B. V. Cheney, *ibid.*, **89**, 5315 (1967).
(49) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).
(50) E. B. Wilson, *Advan. Chem. Phys.*, **2**, 367 (1959).
(51) K. C. Ramey, D. C. Lini, R. M. Moriarty, H. Gopal, and H. G. Welsh, *J. Amer. Chem. Soc.*, **89**, 2401 (1967).
(52) F. R. Jensen and J. J. Miller, *Tetrahedron Lett.*, **40**, 4861 (1966).
(53) E. Crundwell and W. Templeton, *J. Chem. Soc.*, 1400 (1964).
(54) R. M. Moriarty, H. Gopal, H. G. Welsh, K. C. Ramey, and D. C. Lini, *Tetrahedron Lett.*, 4555 (1966).
(55) B. Capon, *J. Chem. Soc.*, 1207 (1971).

Proximity Correction. In order to isolate the factors which might be related to orientation it is necessary to correct for the proximity factor, *i.e.*, the factor involved in the juxtaposition of the reacting atoms exclusive of orientation. In the 2,6 derivative the OH and carboxyl carbon are juxtaposed and therefore no correction need be applied to this compound. The rate of bimolecular reaction was multiplied by 55 (equivalent to 8 eu) to make the rate comparable to the case where the two reacting atoms are juxtaposed as in the *endo*-2,6-norbornane hydroxy acid. (Actually $55/n$ or about 10 would be a more relevant number but the larger value was chosen to minimize objections.) The arguments for the validity of this correction factor have been presented elsewhere.[11,12]

The proximity correction for the 2,3-norbornane derivative and the γ-hydroxybutyric acid can be made in several ways but the simplest method is to compare the number of rotational conformers with juxtaposed reacting atoms with the total number of rotational conformers. If we assume three minima for each carbon–carbon single bond then only one-third of the rotational conformations of the 2,3-norbornane derivative have favorable proximity so a correction factor of 3 was applied. Similarly, two of the nine rotational conformations of γ-hydroxybutyric acid have favorable orientation so a proximity correction of 4.5 was applied to this compound.

Discussion

The relative rates of the various esterification reactions are recorded in Table V normalized to a value of 1.0 for the bimolecular esterification reaction. The measured rates are shown in column 2 and the rates corrected for proximity and torsional strain effects are shown in the last column. The hypothesis of this paper is that these residual rate ratios represent, to a first approximation, the increase in velocity of reaction due to the constrained orientations of the intramolecular systems relative to that of random bimolecular collisions.

Table V. Relative Rates of Acid-Catalyzed Esterifications

Reacting compd	Rel rate[a]	Proximity correction	Torsional strain correction	Corrected rel rate[a]
I	1	55		1
II	80	4.5	64	413
V	6,620	3	4.4	1,660
VI	1,030,000			18,700
III	117	4.5	21.9	200
IV	316	3		17

[a] Normalized to value for bimolecular reaction of EtOH and HOAc. All rates extrapolated to 1 *N* H^+ at 25° in 20% ethanol, $\mu = 0.40$.

The identification of a large factor with the orientation effect depends on the correct assessment of other factors which might contribute to the relative rates of intramolecular and intermolecular reactions. In the following discussion each of these factors will be considered individually.

Proximity. Since the magnitude of the factor identified with orientation depends in part on the proximity

factor and since the latter has been criticized[56-58] it is perhaps desirable to examine its reliability carefully.

The proximity factor was originally derived in order to have some basis for comparing unimolecular reactions in enzyme sites or intramolecular reactions with their bimolecular counterparts. It essentially defined proximity as shown in Figure 1 as the loose complex of reacting molecules in which the reactive portions were juxtaposed but neither bonded to, nor oriented relative to, each other.[11] The factor $55/n$ (where n is the number of nearest neighbors) was obtained as an order of magnitude term for this entropic correction using probability considerations.[11,12] Recently, a similar value was obtained from a statistical mechanical calculation involving a reaction which does not require orientation.[12]

This value for proximity has been criticized on the following grounds: (a) that real molecules will have net attractions or repulsions which are not accounted for in the $55/n$ factor,[56] (b) that proximity should be defined as translational entropy and hence must be greater than $55/n$,[57] and (c) that the empirical finding of $k_{\text{intramol}}/k_{\text{intermol}}$ ratios greater than 55 indicates that the factor is incorrect.[56,58]

In regard to a it was explicitly stated in the derivation that the reacting molecules were assumed to have no net attraction or repulsion to each other or solvent as a way to obtain the idealized entropic value. This is precisely the approach taken in perfect gas theory, Debye–Hückel calculations, etc. Correction factors for deviations from ideality have been discussed qualitatively[11] and quantitatively.[12] The latter leads to the correction factor ψ of eq 8 where K_D is the known dissociation constant for AB molecules in constellations similar to those of Figures 1B and 1C (*cf.* ref 12). Since K_D is rarely known and since many molecules do not seem to have strong attractions or repulsions, *e.g.*, in the case of esterification, the generalized entropic correction is useful in many cases. Obviously where K_D is known or can be calculated the correction factor ψ should be applied.

$$\psi = \frac{K_D + [B]}{54 + [B]} \qquad (8)$$

In regard to b there is, of course, a matter of definition which may depend on individual preferences. Nevertheless, since translational entropy is well known and understood there seems little reason to adopt a new term for the same concept. Moreover, proximity has been widely used in textbooks and elsewhere to mean a loose complex which retains rotational energy and freedom of the individual molecules and of the loose complex, *i.e.*, the state of two molecules in van der Waals contact in an ideal liquid. Translational entropy is an oversimplification of the energy and entropy considerations in forming such a loose complex. Further, the loss of translational entropy in forming the complex of Figure 1A is the same as the loss of entropy in forming the complex of Figures 1B and 1C, yet in the former the reactive groups are not in proximity. Finally, the calculation of the equilibria between forms of the type of Figure 1A and those of 1B and 1C using statistical mechanics is difficult or impossible for large molecules. Thus, the translational entropy approach to proximity, although definable in quantitative terms for single small molecules, will not allow a meaningful separation of factors for most of the compounds of interest to biochemists. Although it is useful and valuable to discuss reactions in terms of translational entropy where these terms are applicable, the concepts of proximity and orientation are distinct and useful and would seem to us more directly applicable to the role of catalysts and model reactions.

In regard to c, the purpose of the proximity calculation is to assign the fraction of an overall acceleration which might be identified with an enforced proximity. If this number is less than the experimental observation, that indicates that other factors, *e.g.*, orientation, may play a role. Thus, the absence of a direct correlation between the proximity factor and intramolecular rates is expected, and perfectly compatible with the derivation of this factor.

It should be emphasized that the important goal is to obtain a rough quantitative estimate for the proximity factor. Two decimal places are not required but an order of magnitude is. If the former were necessary, the number of nearest neighbors would become very important, but since it is known that maximally packed water is only two times as dense as ordinary water, the rough factor for ordinary water is sufficient for present purposes. The same is true of the rest of the arguments and it is believed that a correct order of magnitude figure has been obtained.

The factors of 3.0 and 4.5 for the distribution of conformational isomers in the intramolecular reactions assume that all three conformational minima are equally probable. All three conformational minima will in fact not be equally probable since steric interactions will weight certain conformations. Those conformations which place the hydroxyl and carboxyl groups in maximum proximity may have greater steric repulsion between the hydroxyl and carboxyl groups; however, the same steric repulsions will occur in the bimolecular reactions during bond formation. Thus, as a first approximation, this correction for conformational isomers is probably reasonable.

Torsional Strain. The torsional strain correction has been tested in many compounds and appears to give correct values with reasonable errors for most simple compounds like these. It is to be noted that the correction factors are not large—the largest is 64 for the γ-hydroxybutyric acid and is less than 10 for the other compounds. No correction at all is made for the fastest reacting compound, the 2,6-norbornane derivative. The correction is applied only to the interactions which change during reaction, it being assumed that the fixed part of the norbornane structure remains unchanged to a first approximation. There is, of course, some alteration in the norbornane ring but it should be very small with respect to this correction. This correction will be in some error for the hydroxybutyric acid of course, if the transition state is not chosen correctly, but since that does not affect the conclusions of this article the rough calculation is adequate.

Solvation. It is deduced that no "solvation effect" factor should be used to correct for a presumed differ-

(56) T. C. Bruice, A. Brown, and D. O. Harris, *Proc. Nat. Acad. Sci. U. S.*, **68**, 658 (1971).
(57) M. I. Page and W. P. Jencks, *ibid.*, **68**, 1678 (1971).
(58) T. C. Bruice and A. Turner, *J. Amer. Chem. Soc.*, **92**, 3422 (1970).

ence in environment in the intramolecular reactions relative to the bimolecular reaction. This is based on the following arguments. (a) The acid-catalyzed esterification reaction is by itself insensitive to solvation effects. Studies by Brownstein and Torilla[59,60] have shown relatively small changes in rates of acid-catalyzed esterification as large amounts of organic solvents are added to water. The hydrolysis of ethyl acetate does not seem to be greatly solvent dependent. For example, the rate of ethyl acetate hydrolysis varies less than fourfold in going from pure water to 60% acetone.[61] (b) It has been shown by Bruice that the ratio of intramolecular to intermolecular reaction rates for anhydride formation does not change greatly in going from pure water to 1.0 *M* water in DMSO.[58] (c) In general, increased substitution decreases the rates of acid-catalyzed esterifications.[62] The rates of esterification of methanol by acetic acid, propionic acid, and butyric acid decrease by a factor of 2 and there is no evidence that increasing the general hydrophobicity of the carboxylic acid or alcohol will enhance the rates of esterification. On the contrary, the larger acids and alcohols experience greater steric hindrance in formation of the tetrahedral intermediate and this retards the rates. (d) Neither the supporting ring system nor the juxtaposed hydroxyl groups affected the pK_a values of the various carboxylic acids. Large solvation differences should affect the pK_a values of the carboxylic acids significantly.

Van der Waals Strain. Relief of van der Waals strain or compression is not present in the hydroxybutyric acid, the 2,3-norbornane derivative, or the benzoic acid derivative as there is free rotation which would allow relief from such strain in the ground state. Compression could be present in the 2,6-norbornane derivative but the equilibrium constant for the endo–exo equilibrium described above either indicates it is absent or compensated for by some other factor. It will therefore not serve as an explanation for the high rates of any of the intramolecular reactions.

Ring Strain. Ring strain is also a possibility. Such an effect in the intramolecular reactions will lower their rates relative to the bimolecular reaction and therefore would, if present, tend to increase the factor identified with orbital steering. It could, however, change some of the relative rates between the various intramolecular compounds. The 2,6-norbornane lactone has approximately conventional bond angles and lengths as does the 2,3 derivative. The γ-hydroxybutyric acid can adopt normal angles, etc. The alkaline hydrolysis rates which are extraordinarily similar for all of the lactones and the infrared stretching frequencies are further indications of little ring strain in the lactones. Thus, the factors identified with orbital orientation might be changed slightly relative to each other but would, if anything, be increased if ring strain is involved in the formation of any of the intramolecular compounds.

The fact that norbornane rings are used may raise the question of the complexity of nonclassical ion behavior or strain in bond angles of the norbornane system. However, the norbornane system is used only as a superstructure to which the lactone ring system is attached. The reactions involve classical esterification and the COOH and OH groups which participate are appended to, and are not internal to, the norbornane system.

Inductive Effects. Electronic or inductive effects were deliberately minimized and are essentially identical in all of the saturated rings. The benzoic acid derivative has a double bond to transmit resonance effects and therefore its rates are not used in any of the arguments for the orientation effects. The alkyl groups are well known for small inductive effects and the effects are dissipated rapidly in a saturated ring system.[63] Therefore, very small, if any, inductive effects should be present. This is also indicated by the small p*K* differences in the carboxylic acids.

Ground State *vs.* Transition State. An interesting relationship arises from the equilibrium studies. In most acid-catalyzed esterification reactions, the overall equilibrium constant remains quite the same even though the forward and reverse rates vary enormously with substituents.[64] In the present case there is a rough, but not exact, parallel between the forward rates and the equilibrium constants for the lactonizations. This may suggest that the rates are caused by a selection of ground states in the open-chain analogs for those geometries which react most readily. The fact that there is not an exact parallelism (and in fact the equilibrium constant ratios can vary by a factor of 20 in some cases from the rate ratios) indicates that the ground-state selection is not the only factor. However, the results suggest that the confined geometry of the intramolecular compounds has selected those ground states which are already in a highly favorable geometry or those which can readily assume a highly favorable transition-state geometry.

The smaller change in the reverse direction would arise from the similar structure of all the lactones which, as discussed above, will assume similar bond angles and confined structures by the inherent limitations of the five-membered rings. There is also some indication that five-membered lactone rings are already in a favorable geometry for nucleophilic attack as indicated by their increased susceptibility to basic hydrolysis relative to the basic hydrolysis of ethyl acetate.

General Comments. The analysis in this article leads to the conclusion that factors of 10^4 can be identified with preferred orientations of reacting atoms relative to the chance encounter of a bimolecular reaction. This conclusion has led some to deduce[56] that extremely small angles of approach, *i.e.*, 0.1°, of reacting atoms would be required in order to achieve such factors.

If two reacting atoms can only react over some fraction of their total solid angles $\Delta\phi$, $\Delta\theta$ of Figure 4 then the probability that any random collision has proper orientation is given by $\Delta\phi^4/4\pi^2$ (assuming $\Delta\theta_1 = \Delta\phi_1 = \Delta\theta_2 = \Delta\phi_2 = \Delta\phi$). The orientation factor of

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(63) H. C. Brown, D. H. McDaniel, and O. Haflinger in "Determination of Organic Structures by Physical Methods," E. A. Baude and F. C. Nachod, Ed., Academic Press, New York, N. Y., 1955, pp 567–662.
(64) G. E. Branch and D. S. McKittrick, *J. Amer. Chem. Soc.*, **45**, 321 (1923); H. A. Smith and J. H. Steele, *ibid.*, **63**, 3466 (1941); H. S. Levenson and H. A. Smith, *ibid.*, **62**, 1556 (1940).

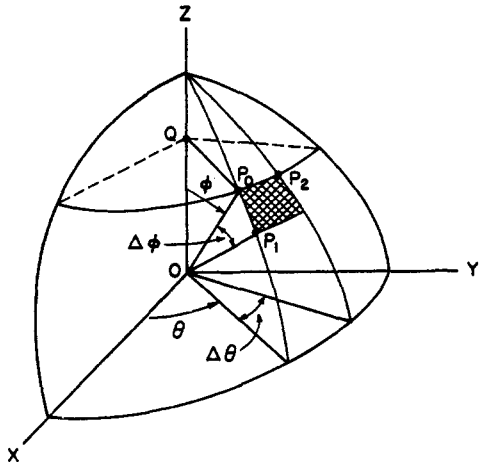

Figure 3. A hypothetical reactive portion of an atomic surface defining the angles $\Delta\theta$ and $\Delta\phi$.

10^4 can, in that case, be achieved by two-dimensional reactivity windows of 10°. (Such a calculation applies to Figure 3 but would have to be modified in specific cases. Nevertheless it gives a rough indication of geometry requirements.) This is not an unreasonable number even considering conventionally accepted bending vibration amplitudes. Statistical mechanical calculations indicate that factors of this sort are acceptable with known values for such terms. What is forgotten in criticism of these numbers is the added entropy effects acquired once orientation is needed. In Figure 1B are seen two reactants at an angle which is not optimal, whereas in Figure 1C these same reactants are optimal. The energy and entropy necessary to align the reactants can be achieved by binding to an enzyme surface or by a predesigned organic superstructure. However, the increase in rate beyond the proximity effect will be small if the reactants have no orientational requirement. The factor of 10^4 therefore derives in part from the size of the window allowing reaction and in part from the entropic requirements for rotating the molecules into an alignment consistent with these reactivity "windows."

The fact that these numbers in these first examples are compatible with conventional calculations is reassuring but should not obscure the potential for a confrontation between current theory and experiments of this sort. If indeed an optimal arrangement has been achieved in the 2,6-norbornane derivative then 10^4 can be rationalized on the basis of current statistical mechanics. If, as seems likely, the first series of compounds of this sort has not achieved an optimal orientation then larger factors may arise in the future. Such larger numbers could not be considered impossible theoretically simply because we need make so many assumptions in theoretical calculations. It is, in fact, not known how sensitive the transition state is to orientation, how much the bimolecular reaction is subject to suboptimal orientations, how far gas-phase calculations can be applied to solution, etc. These experiments have focused on experimental devices for obtaining a number for the orientation factor in reaction rates, and the experimental results are more convincing than any theoretical arguments in such complex systems.

In a given intramolecular reaction the acceleration (or deceleration) resulting from orbital steering of the reactants will depend on the steric requirements of the two atoms involved. In one enzyme the simultaneous or near-simultaneous reaction at several loci may be involved (*cf.* Figure 2). The combination of factors of 10^4 at several such loci can then give very large accelerations (*e.g.*, $10^4 \times 10^4 \times 10^4$) and this ability to optimize orientation of reacting orbitals may be a major factor in the special catalytic power of enzymes.