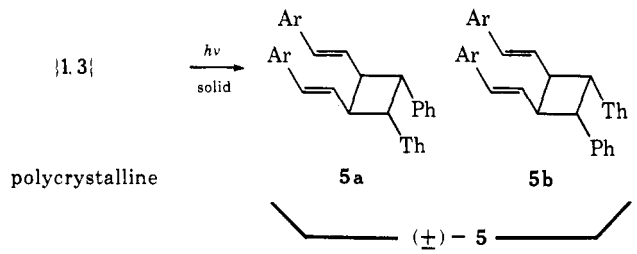
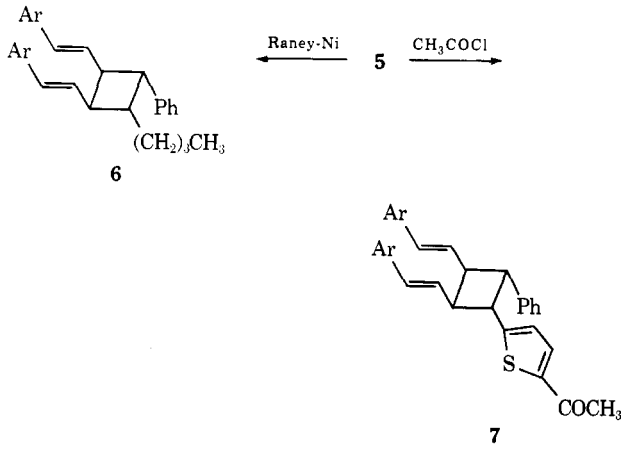


We wished to establish whether photoexcited molecules of **3** contained in stacks of **1** would exhibit differences in reactivity of their "upper" and "lower" faces during reaction (Figure 1) to give **5a** and **5b**, respectively. (The crystal structure of **1** has been determined in this laboratory[3,8] and Figure 1 was constructed by simply substituting a thiophene for a phenyl within the stacks of **1**. The ground-state perturbation to translational symmetry produced by inclusion of guest molecules of **3** is, we believe, small and the ratio of **5a** to **5b**, *i.e.*, the optical yield of the reaction, will primarily reflect the nature of the excited state of **3** responsible for cyclodimerization.) For this purpose we prepared, by growth from the melt in evacuated glass bulbs,[9] large (1–6 g) *single* mixed crystals containing 15% of **3** in **1**, which were powdered and irradiated as above. The resulting mixed dimer **5** was consistently found to possess optical activity, some crystals affording dextrorotatory and some levorotatory material, $[\alpha]_D$ + or − *ca.* 1°.[10] Transformation of optically active **5** to a less symmetrical molecule led to many-fold enhancement of specific rotation. Raney nickel reductive degradation of the thiophene group in (+)-**5** [or(−)-**5**] led to (−)-**6** [or (+)-**6**], $[\alpha]_D$ *ca.* 10°,[11] while acetylation of (+)-**5** afforded (+)-**7**,[12] $[\alpha]_D$ *ca.* 5°.



6

7

(8) We thank Dr. D. Rabinovich and Z. Shaked for providing us with the crystal structure data for **1**. A table of atomic coordinates will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N.W., Washington, D. C. 20036, by referring to code number JACS-73-2058. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche.

(9) J. N. Sherwood, and S. J. Thomson, *J. Sci. Instrum.*, 37, 242 (1960). We are most grateful to Dr. Joel Bernstein, Professor M. D. Cohen, and Z. Ludmer for help in constructing this apparatus and for much useful advice.

(10) Measured on a Perkin-Elmer Model 141 polarimeter in chloroform with 1-ml, 1-dm cuvettes. Values for α_{obsd} (mg of **5**): 0.076° (67), 0.070° (66), −0.076° (100).

(11) Colorless oil, *m/e* 528 (M^+, Cl_4), 368 [$C_6H_3Cl_2(CH{=}CH)_2C_6H_3Cl_2$, 3%], 274 [$C_6H_5(CH{=}CH)_2C_6H_3Cl_2$, 80%], 254 [$C_6H_3Cl_2(CH{=}CH)_2C_4H_9$, 100%], 160 [$C_6H_3CH{=}CHC_4H_9$, 48%].

(12) Almost colorless solid, mp 121–122°; nmr: $\delta_{TMS}^{CDCl_3}$ 2.4 (3 H, s, CH_3), 3.8–4.3 (4 H, m, cyclobutane CH), 6.5 (4 H, m, =CH—), 7.2 (13 H, m, aromatic).

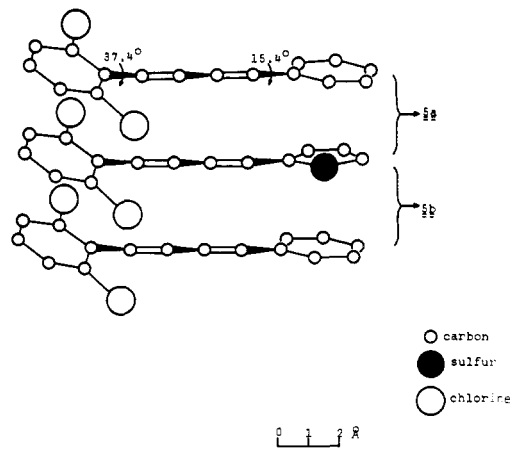


Figure 1. Packing diagram of a mixed crystal of **1** and **3**, based on the crystal structure of **1**[3,8] showing the intermolecular contacts which lead to the enantiomeric photodimers **5a** and **5b**.

Currently, the optical yield of this reaction is being established and the absolute configuration of a given single mixed crystal (*i.e.*, whether the stacks of molecules are twisted as in Figure 1 or as in its mirror image) and of the dominant cyclobutane photoproduct (*i.e.*, **5a** or **5b**) from that crystal, or one having the same handedness, are being determined. When complete, this information will indicate which face of a diaryl butadiene of structure **1** or **3** is the more reactive one in this system and will provide insight into the reaction coordinate as ground-state molecules, initially separated by 4 Å, move to a final bonding distance of 1.5 Å.

Finally, the simple production of stable, optically active samples from optically inactive starting material is highly relevant to current hypotheses on the prebiological origin of optical activity on earth.[13]

Acknowledgment. We thank Professors M. D. Cohen and F. L. Hirshfeld for valuable discussions, and Dr. D. Rabinovich and Z. Shaked for the crystallographic data. We are grateful for a MINERVA Grant for partial support of this research.

(13) We note that the use of single crystals is not essential for the success of these experiments. Irradiation of polycrystalline samples prepared by slow evaporation of an ethanolic solution of **1** and **3** also affords optically active **5**, albeit of lower specific rotation. This is apparently due to self-innoculation by a relatively small number of initial nuclei. Rapid formation of mixed crystals of **1** and **3** generally led to racemic **5**.

(14) Ph.D. student of the Feinberg Graduate School.

(15) Deceased July 12, 1971.

Asher Elgavi,[14] Bernard S. Green,* G. M. J. Schmidt[15]
Department of Structural Chemistry
The Weizmann Institute of Science, Rehovot, Israel
Received October 13, 1972

General Base Catalyzed Intramolecular Transesterification

Sir:

The α-chymotrypsin-catalyzed hydrolysis of esters and amides involves acylation of serine-195, with release of the alcohol or amine portion of the substrate, followed by deacylation to regenerate active enzyme.[1] The generally accepted mechanism for the

(1) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. I, W. A. Benjamin, New York, N. Y., 1966, Chapter 2; M. L. Bender, "Mechanisms of Homogeneous Catalysis from Protons to Proteins," Wiley, New York, N. Y., 1971.

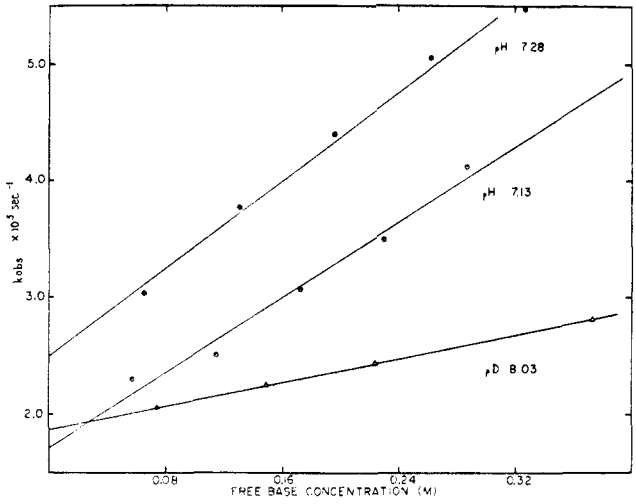


Figure 1. Plot of k_{obsd} *vs.* imidazole free base concentration (M) for cyclization of ethyl 2-hydroxymethylbenzoate to phthalide at 30° in H_2O or D_2O (Δ) with $\mu = 0.5$ (held constant with KCl).

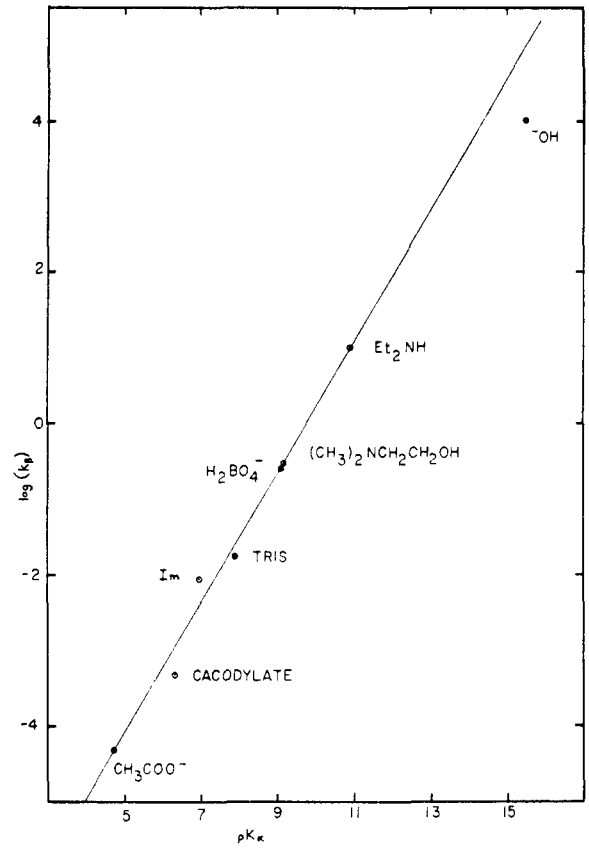


Figure 2. Bronsted plot of log k_B *vs.* the pK_a of the catalyzing base in the cyclization of ethyl 2-hydroxymethylbenzoate to phthalide at 30° in H_2O ($\mu = 0.5$).

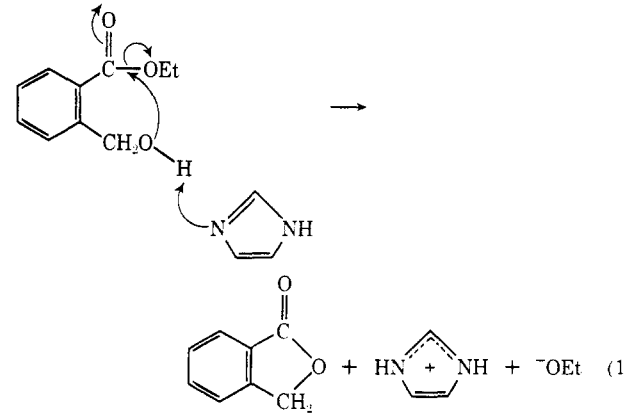
acylation step involves histidine-57 functioning as a general base, partially abstracting a proton from the serine hydroxyl as it attacks the carbonyl group of the substrate.[1]

Neighboring phenoxide and alkoxide ions have been studied as intramolecular nucleophiles in reactions of substituted phenyl and ethyl carbamate esters.[2] These reactions are very efficient, and effective molarities for the neighboring group of 10^6–10^8 M can be calculated in comparison with analogous bimolecular reactions. Thus, a neighboring oxide ion is a powerful intramolecular nucleophile in reactions at the ester carbonyl.

A neighboring hydroxymethyl group has been studied as a participant in the hydrolysis of amides.[3,4] In hydrolysis of γ-hydroxybutyramide an accelerated rate is found in the alkaline and neutral pH regions in comparison with acetamide and butyramide.[3] These reactions probably involve attack of the oxyanion on the neutral and protonated amide, respectively. Buffer catalysis is observed in the cyclization of 2-hydroxymethylbenzamide.[4] Both general base and general acid catalysis by imidazole were reported. There have not previously been any studies of general base catalyzed transesterification reactions of esters analogous to the chymotrypsin acylation step. We wish to report the finding of such catalysis in the cyclization of ethyl 2-hydroxymethylbenzoate (I) to phthalide.

Ethyl 2-hydroxymethylbenzoate was prepared by reaction of 2-hydroxymethylbenzoic acid with triethyloxonium fluoroborate. The rate of cyclization of I was measured spectrophotometrically with a Gilford 2000 recording spectrophotometer or Durrum Model D-110 stopped-flow spectrophotometer by following the change in absorbance at 254 nm, due to formation of the product phthalide, in H_2O as the solvent and at 30°. Phthalide is stable under the experimental conditions. Ionic strength was maintained constant at 0.5 with KCl. Ethyl 2-hydroxymethylbenzoate was introduced into the reaction cuvette (3 ml of buffer) as a solution in 20 μl of acetonitrile. The final concentration of acetonitrile was therefore 0.6%. Observed pseudo-first-order rate constants were calculated with an IBM 360-50 computer.

Imidazole is a good catalyst for the cyclization reaction as seen in Figure 1, where k_{obsd} is plotted *vs.* imidazole free base concentration. The parallel lines at two pH values show that the base form of imidazole is the catalyst. Thus, there is a first-order dependence on imidazole free base concentration in the reaction. The value of k_{Im}, the second-order rate constant, is 8.74×10^{-3} M^{-1} sec^{-1}. Rate constants were also obtained in D_2O as the solvent, the value of $k_{Im}^{H_2O}/k_{Im}^{D_2O}$ being 3.46. It is probable therefore that the mechanism of the reaction involves general base catalysis or a kinetic equivalent as in eq 1.



(1)

Other bases will also catalyze the cyclization reaction. In Figure 2 is shown a plot of the logarithms of

(2) J. E. C. Hutchins and T. H. Fife, *J. Amer. Chem. Soc.*, in press.
(3) T. C. Bruice and F. H. Marquardt, *ibid.*, **84**, 365 (1962).
(4) C. J. Belke, S. C. K. Su, and J. A. Shafer, *ibid.*, **93**, 4552 (1971).

the second-order rate constants *vs.* pK_a of the catalyzing base. The Brønsted coefficient is 0.87. In cyclization reactions of carbamate esters having a neighboring hydroxymethyl group, buffer catalysis is not observed, the reaction involving preequilibrium ionization of the hydroxymethyl group.[2] This is most likely due to the presence of a deactivated acyl group necessitating nucleophilic attack by a fully developed negative charge. With I, however, the acyl group is not as deactivated, and proton transfer is not complete in the critical transition state, although the Brønsted coefficient of 0.87 indicates that proton transfer is appreciable.

Extrapolation of the plots of k_{obsd} *vs.* buffer concentration to zero buffer concentration gives values of the rate constants for spontaneous cyclization. The spontaneous reaction is subject to hydroxide and hydronium ion catalysis with $k_{OH} = 10^4\ M^{-1}\ sec^{-1}$ and $k_{H^+} = 3.35 \times 10^{-3}\ M^{-1}\ sec^{-1}$. Thus, the spontaneous reaction itself is a relatively facile process. In comparison, ethyl esters normally require high pH and elevated temperatures for rapid hydrolysis. In the case of ethyl benzoate, k_{OH} is $3.0 \times 10^{-2}\ M^{-1}\ sec^{-1}$ at 25° in H_2O.[5] Therefore, k_{OH} for I is approximately 10^5 greater.

It can be concluded that a neighboring hydroxymethyl group is a powerful intramolecular nucleophile in transesterification reactions even when proton transfer is only partial. The reaction of eq 1 is very likely closely analogous to the reaction of α-chymotrypsin with ethyl esters. Belke, *et al.*,[4] concluded that imidazole-catalyzed cyclization of 2-hydroxymethylbenzamide to phthalide is a good model for acylation of α-chymotrypsin by amide substrates. The present findings with ethyl 2-hydroxymethylbenzoate now make derivatives of 2-hydroxymethylbenzoic acid reasonable models for reaction of α-chymotrypsin with both ester and amide substrates.

Acknowledgment. This work was supported by a research grant from the National Institutes of Health.

(5) M. L. Bender, *J. Amer. Chem. Soc.*, 73, 1626 (1951).
(6) National Institutes of Health Predoctoral Fellow.

Thomas H. Fife,* Bruce M. Benjamin[6]
Department of Biochemistry, University of Southern California
Los Angeles, California 90033
Received November 13, 1972

Hyperfine Interactions in Perturbed Nitroxides

Sir:

The stability of aliphatic nitroxides, the simplicity of their epr spectra, and, in particular, the sensitivity of their epr spectra to environmental perturbations have made them objects of considerable interest and wide application.[1] In this work we have measured the spin-Hamiltonian parameters for di-*tert*-butyl nitroxide (DTBN) and 2,2,6,6-tetramethylpiperidine-*N*-oxyl (TMPN) perturbed by molecular complex formation, by H bonding, and by solvent effects in aprotic solvents. These perturbations cause a rearrangement of the π-electron charge and spin density, increasing spin density in the 2pπ orbital of nitrogen (ρ_N^π) and altering the spin-Hamiltonian parameters.[2] From these measurements we obtain the Karplus–Fraenkel parameters,[3] Q_N and Q_{ON}^N, which relate the nitroxide ^{14}N isotropic splitting constant to ρ_N^π and ρ_O^π, respectively (eq 1).

$$a_N = Q_N\rho_N^\pi + Q_{ON}^N\rho_O^\pi \quad (1)$$

We have also prepared a new series of free-radical molecular complexes,[2] between DTBN or TMPN and the Lewis acids MCl_4 (M = Si, Ge, Sn, or Ti) and $Ti(OPh)_4$.[4] Although TMPN is oxidized by $SnCl_4$ at room temperature,[5] complexation is observed at low temperature.

In fluid solutions, isotropic splitting constants are observed for ^{14}N, as well as for nitroxide ^{13}C and for ^{119}Sn–^{117}Sn in the $SnCl_4$ complex.[4] In frozen nitroxide solutions the epr spectra are dominated by the anisotropic ^{14}N hyperfine splitting. These powder spectra were interpreted on the basis of the spin Hamiltonian[6]

$$\mathcal{H} = \beta S\cdot\mathbf{g}\cdot H + A_N S_z I_z + B_N(S_x I_x + S_y I_y) \quad (2)$$

using appropriate spectral features in conjunction with computer simulations.[4] A_N gives rise to well-resolved splittings and is obtained with good accuracy, but the splittings due to B_N are not resolved. We are interested in T_N, the anisotropic (dipolar) hyperfine splitting (hfs) constant; since $A_N = a_N + T_N$ and $B_N = a_N - T_N/2$,[7] T_N was obtained from values of a_N and A_N measured from fluid and frozen solutions, respectively. This procedure was verified by simulating the powder spectra with the measured A_N and a calculated value of B_N.

Figure 1 gives a plot of T_N *vs.* a_N for DTBN and TMPN perturbed by a variety of interactions. Included are the results for the newly prepared free-radical molecular complexes.[4] With the exclusion of protonated DTBN (G and H), there is a linear relationship between T_N and a_N. This linear variation includes perturbation by "simple" solvent effects involving non-hydrogen-bonding solvents, points a–c, perturbation by hydrogen-bond formation, points 1–7, and also by actual molecular complex formation, points A–F. These results are similar to previous observations that variations of hfs constants are linearly related for a radical whose spin density distribution is perturbed by intermolecular interactions without structural changes in the radical.[10]

A linear least-squares fit of data for both radicals gives the straight line in Figure 1

$$a_N(G) = (0.61 \pm 0.02)T_N + (3.6 \pm 0.4) \quad (3)$$

where the errors given are the probable errors in the slope and intercept. Because we include results from

(1) For some recent reviews, see: (a) E. G. Rozantsev, "Free Nitroxyl Radicals," Plenum Press, New York, N. Y., 1970; (b) P. Jost and O. H. Griffith, *Methods Pharmacol.*, 2, 223 (1972).
(2) T. B. Eames and B. M. Hoffman, *J. Amer. Chem. Soc.*, **93**, 3141 (1971).
(3) M. Karplus and G. K. Fraenkel, *J. Chem. Phys.*, **35**, 1312 (1961).
(4) A. H. Cohen and B. M. Hoffman, manuscript in preparation.
(5) Y. Takaya, G. Matsubayashi, and T. Tanaka, *Inorg. Chim. Acta*, **6**, 339 (1972).
(6) L. J. Libertini and D. H. Griffith, *J. Chem. Phys.*, **55**, 1359 (1970).
(7) These equations do not include the dipolar interaction between spin density on oxygen and the ^{14}N nucleus. This quantity, which can be calculated from the equations of McConnell and Strathdee[8] as modified by Barfield[9] using a self-consistently calculated value of ρ_O^π, is small ($0.66\rho_O^\pi$ G) for our perturbed nitroxides and is included in the data in Figure 1.
(8) H. M. McConnell and J. Strathdee, *Mol. Phys.*, **2**, 129 (1959).
(9) M. Barfield, *J. Chem. Phys.*, **53**, 3836 (1970).
(10) A. H. Reddoch, *ibid.*, **43**, 225 (1965).