Analysis of Solvent Effects on the Decarboxylation of Benzisoxazole-3-carboxylate Ions Using Linear Solvation Energy Relationships: Relevance to Catalysis in an Antibody Binding Site

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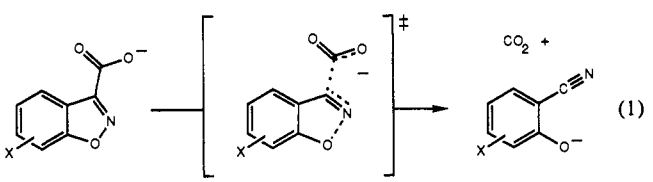
Abstract: The mechanisms by which specific solvent properties influence the title reaction, which is extremely medium-sensitive, have been investigated using multiparametric methods. The results of this analysis have been compared with previous experimental studies of the reaction mechanism. Hydrogen-bond donation by solvent and hydrogen-bond donation by tetramethylguanidinium ion in tight ion pairs with the carboxylate greatly retard the reaction. Solvent dipolarity and basicity accelerate the reaction, most likely by helping to break up hydrogen-bonded ion pairs. The rate of decarboxylation in the binding pocket of a catalytic antibody developed for this reaction is slower than that expected of a free carboxylate in an aprotic environment. Therefore, the binding site may contain a hydrogen-bond-donating species. A better catalyst might be developed by approaches that reduce hydrogen bonding in the active site without reducing substrate binding.

Introduction

The rates at which benzisoxazole-3-carboxylate ions decarboxylate (eq 1) are strongly dependent on the reaction medium.



In solvent-effect studies, Kemp and Paul showed that the first-order rate varies by up to 8 orders of magnitude on going from reaction in water to reaction in dipolar aprotic solvents such as *N,N*-dimethylformamide and hexamethylphosphoramide.[1-4] These results are reproduced in the first two columns of Table I. Rate accelerations (relative to water) have also been observed in studies using a variety of other media, including micelles, bilayers, macrocyclic hosts, and polymers.[5-15] Certain polymers can also accelerate the reaction in organic solvents.[16,17] Recently, it has been shown that the reaction can be accelerated by a factor of 19 000 (relative to water) in the binding pocket of a catalytic antibody.[18]

Kemp's studies were clear in showing that the reaction rate is retarded by solvents that can hydrogen bond to the reactant carboxylate.[1-4] Decarboxylation rates are slowest in protic solvents such as water and alcohols. Moreover, the presence of an intramolecular hydrogen bond in 4-hydroxybenzisoxazole-3-carboxylate, **1**, results in slow decarboxylation rates in all solvents. Rate data for this compound in various solvents are included in Table II. On the other hand, meaningful trends among solvents producing intermediate rates are not immediately obvious because solvents with very different properties sometimes give similar rates. Carbon tetrachloride, ethanol, and formamide all yield similar rates, even though these vary widely in dipolarity, hydrogen-bond basicity, and hydrogen-bond acidity.

On the basis of solvent-extraction studies, Kemp speculated that dispersion interactions might stabilize the polarizable charge-delocalized transition state.[2] Effective stabilization would require that polarizable portions of the transition state be precisely matched with complementary polarizable regions of the solvent. In principle, such interactions might account in part for rate variations observed in aprotic solvents, where increasing rates are observed with increasing solvent dipolarity. However, the observed trend in this reaction runs contrary to the Hughes–Ingold rules for medium effects on a reaction proceeding from

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a charged reactant to a charge-delocalized transition state.[19] The rate of such a reaction is usually expected to be slower in more polar solvents because a localized charge is more readily stabilized by neighboring dipoles than a delocalized charge.

Ion-pairing interactions between the carboxylate and its counterion further complicate interpretation of the decarboxylation rate data. The reactant carboxylate was generated from the corresponding acid by reaction with tetramethylguanidine (TMG), generating tetramethylguanidinium ion ($TMGH^+$) as the counterion.[1,3] In aprotic solvents of low to medium polarity, Kemp noted that $TMGH^+$ is likely to be ion-paired with the carboxylate.[1] Therefore, the observed decarboxylation rates do not necessarily represent the rates of decarboxylation of the free carboxylate ion but should instead be regarded as lower limits for these rates.

Smid and co-workers have suggested that such ion-pairing effects, rather than dispersion interactions between the solvent and the transition state, might account more generally for the rate trends observed in aprotic solvents.[20] They had observed that the rate of decarboxylation in benzene with a cryptated potassium ion as the counterion was nearly 1000 times faster than the rate observed in benzene with $TMGH^+$.[17] They also studied the spontaneous decomposition of 6-nitrobenzisoxazole-3-carboxylic acid in ethers. On the basis of the inhibitory effects of added *p*-toluenesulfonic acid, this reaction was postulated to occur via very low concentrations of free carboxylate in equilibrium with the carboxylic acid in the ethereal solvents.[21] The rate constant for decarboxylation of the free ion in tetrahydrofuran calculated from this model was greater than the rate constant found previously for the $TMGH^+$ salt in hexamethylphosphoramide, the most accelerating solvent in Kemp's original data set.[22] These results are thus consistent with the expectation that free-ion rate constants for a reaction involving a charge-delocalized transition state should be higher in less polar solvents.[23]

Our interest in these solvent effects stems from a desire to better understand the mechanism by which a catalytic antibody for this reaction accelerates the rate.[18] Medium effects must be involved because the reaction is insensitive to general acid–general base catalysis and is not subject to stereochemical constraints. A clearer understanding of solvent effects could shed light on the properties of the catalytic binding site that influence the reaction rate and, more generally, the role of medium effects in enzymatic catalysis. Since the observed decarboxylation rates in solution appear to be dependent on a combination of solvent properties, we have investigated the results in Kemp's data set using the multiparameter methods developed by Kamlet, Abraham, and Taft.[24-27] These methods can help to unravel the complexity of multiple solvent effects occurring simultaneously.

Our analysis indicates that hydrogen-bond donation by either the solvent or $TMGH^+$ greatly retards the reaction under Kemp's experimental conditions. The antibody must catalyze the reaction by reducing hydrogen-bond donation to the substrate when it is transferred from water to the binding site. However, the bound substrate does not appear to be entirely free of hydrogen bonds, because the observed rate acceleration is less than that expected of a completely free carboxylate in an aprotic environment. These studies suggest that the binding site may contain a hydrogen-bond-donating species and that efforts to improve the catalyst should focus on changing the catalyst structure to reduce hydrogen bonding in the active site, while maintaining adequate substrate binding.

Results

Method. In the Kamlet–Abraham–Taft methodology,[24-26] solvent-dependent properties are correlated against a set of solvent parameters by the method of multiple linear regression to yield linear solvation energy relationships of the form:

$$\mathrm{SDP} = \text{constant} + (s\pi_1^* + d\delta) + a\alpha_1 + b\beta_1 + h(\delta_H^2)_1 \quad (2)$$

The solvent-dependent property SDP, such as a rate constant or equilibrium constant, is modeled as the linear combination of a polarity term ($s\pi_1^* + d\delta$),[28] a hydrogen-bonding term in which the solvent is the hydrogen-bond acid ($a\alpha_1$), a hydrogen-bonding term in which the solvent is the hydrogen-bond base ($b\beta_1$), and a cavity term ($h(\delta_H^2)_1$.)

The parameters π_1^*, α_1, and β_1 are known as solvatochromic parameters because they were originally derived using spectroscopic measurements.[30-34] α_1 and β_1 reflect the solvent hydrogen-bond acidity and basicity, respectively.[31,32,35,36] π_1^* indicates the ability of the solvent to stabilize a neighboring charge or dipole; it represents a combination of solvent dipolarity and polarizability.[33,37-39] For simple aliphatic solvent molecules with a single dominant dipole, π_1^* is proportional to molecular dipole moments. In practice, it has been found that polar interactions are best modeled by a π_1^*-term that is adjusted with a polarizability correction factor, as in ($s\pi_1^* + d\delta$), where the value of δ is 1.0 for aromatic solvents, 0.5 for polychlorinated aliphatic solvents, and 0.0 for nonchlorinated aliphatic solvents.[34,38,40] Cavity effects are modeled using the solvent parameter $(\delta_H^2)_1$, which is based on the Hildebrand solubility parameter δ_H.[25,26,37] The square of the Hildebrand solubility parameter gives the solvent cohesive energy density. Cavity effects reflect the energetic cost of disrupting solvent/solvent interactions to create or expand a cavity in the solvent.

The coefficients in each term of eq 2 indicate the extent to which the solvent-dependent property being examined depends on the solvent property represented by the corresponding solvent parameter. The constant in eq 2 arises from the method of multiple

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(22) In making comparisons to his results obtained at 20 °C, Smid applied a temperature correction to Kemp's data collected at 30 °C.
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Table I. Decarboxylation Rates in Various Solvents and Parameters To Describe the Properties of Those Solvents

solvent	log k^a	π_1^*	δ	α_1	β_1	$(\delta_H^2)_1$
water	−5.13	1.09	0.00	1.17	0.47	5.490
methanol	−3.60	0.60	0.00	0.93	0.62	2.052
formamide	−3.13	0.97	0.00	0.71	0.60	3.617
chloroform	−3.09	0.58	0.50	0.44	0.00	0.887
ethanol	−3.00	0.54	0.00	0.83	0.77	1.621
carbon tetrachloride	−2.82	0.28	0.50	0.00	0.00	0.738
benzene	−2.32	0.59	1.00	0.00	0.10	0.838
dioxane	−1.39	0.55	0.00	0.00	0.37	1.000
dichloromethane	−1.33	0.82	0.50	0.30	0.00	0.977
diethyl ether	−1.05	0.27	0.00	0.00	0.47	0.562
nitromethane	−0.24	0.85	0.00	0.22	0.25	1.585
benzonitrile	0.40	0.90	1.00	0.00	0.37	1.229
acetonitrile	0.46	0.75	0.00	0.19	0.37	1.378
tetrahydrofuran	0.60	0.58	0.00	0.00	0.55	0.864
dimethyl sulfoxide	1.00	1.00	0.00	0.00	0.76	1.688
acetone	1.38	0.71	0.00	0.08	0.48	0.906
N,*N*-dimethylforamide	1.56	0.88	0.00	0.00	0.69	1.389
N,*N*-dimethylacetamide	2.20	0.88	0.00	0.00	0.76	1.166
N-methylproline	2.40	0.92	0.00	0.00	0.77	1.276
hexamethylphosphormaide	2.80	0.87	0.00	0.00	1.05	0.734

[a] Rates for the decarboxylation of 6-nitro-3-carboxybenzisoxazole from ref 1.

linear regression. For solvents with $\pi_1^* = \delta = \alpha_1 = \beta_1 = 0$, such as an alkane, the values of the constant and the cavity term give the value of the solvent-dependent property in that solvent. The Kamlet–Abraham–Taft parameters and multiple linear regression methodology have been used to correlate and rationalize solvent effects on a wide variety of properties and processes, including solubilities, partition coefficients, reaction rates, UV–vis spectra, and NMR spectral shifts.[24-27] In many cases, not all of the terms in eq 2 are important to the process being correlated and simpler equations with fewer parameters can be obtained.

Regression Results. The Kemp data set on the decarboxylation of 6-nitro-benzisoxazole-3-carboxylate provides reaction rates in a large variety of solvents.[1] Of the 24 solvents in the set, the relevant solvent parameters are available for 20; these are listed in Table I. Each solvent property varies over a wide range. Simultaneously, the rates vary by nearly 8 orders of magnitude. We set out to investigate how specific solvent properties influence the decarboxylation, using the multiparameter methods described above as tools to extract this information from the observed rates. Regressing the full data set of 20 solvents (Table I) against all five explanatory parameters yielded eq A5, the first equation in Table III. This correlation provides a good fit to the data with no major outlying points, placing all the data on a single line (Figure 1a). From the point of view of predicting reaction rates in new solvents, this result represents a significant advance.[41]

Previous attempts to correlate the observed rate data with solvatochromic parameters E_T and Z were unsuccessful.[1,3] The reaction system, involving both ion pairing and decarboxylation, is presumably too complex to be modeled with a single parameter. In addition, the E_T and Z parameters are not simple measures of solvent polarity. The E_T parameters can be correlated with a linear combination of π_1^*, the δ polarizability correction factor, and α_1, indicating that the solvatochromic model is sensitive to a combination of solvent dipolarity, polarizability, and hydrogen-bond acidity.[34,35,42] The Z scale also involves both solvent polarity and hydrogen-bond acidity effects.[35] We have found that the H solvatochromic parameter described by Kemp and Paul similarly depends on several solvent properties.[1,3] This parameter scale was based on the effects of the test solvents on the UV spectra of a decarboxylation product, 2-cyano-5-nitrophenolate. Regressing this parameter against the Kamlet–Taft explanatory variables for the 20 solvents in the complete data set gives the equation below with $R = 0.968$:

$$-H = -71.02 + (11.55\pi_1^* - 1.79\delta) - 5.92\alpha_1 + 3.38\beta_1 - 1.54(\delta_H^2)_1 \text{ kcal} \quad (3)$$

It is apparent that the H parameter depends on the various solvent properties in a similar manner to the reaction rate (compare eq A5), justifying its consideration as an empirical model for correlation purposes. However, the model does not elucidate the mechanism(s) of this reaction's solvent sensitivity because the H parameter represents a complex combination of solvent properties.[43]

Although the five-parameter equation (A5 in Table III) accounts for all the data in the solvent set, it is often possible in practice to obtain equations with a smaller number of explanatory parameters through judicious selection of solvent subsets. In the present instance, the observed rate data reflect both ion-pairing equilibria that determine the amount of free carboxylate present in the system and relative energies of the reactant carboxylate and charge-delocalized transition state. Each of these processes may be influenced by the solvent separately. We set out to reduce the influence of ion-pairing equilibria on our regression results by removing those solvents with the least ability to solvate and

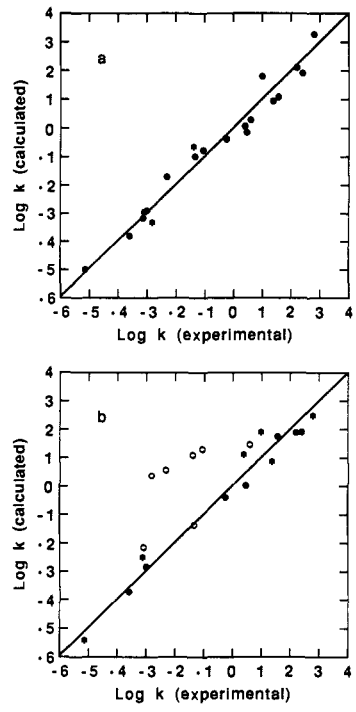

Figure 1. Plots of calculated rates against experimental rates for (a) eq A5 and (b) eq C2. In both plots, solid circles represent solvents included in the solvent set regressed to obtain the equation. In plot b, the open circles represent solvents not included in the solvent set for the regression but calculated with the resulting equation. The four solvents with the largest deviations above the regression line in plot b are, from left to right in the plot, carbon tetrachloride, benzene, dioxane, and diethyl ether.

(41) Plotting the solvent-dependent rates of the other substituted 3-carboxybenzisoxazoles against those of 6-nitro-3-carboxybenzisoxazole yields a set of nearly parallel lines, indicating that the mechanism does not change with substituent, and conclusions we draw on the basis of an analysis of the data for 6-nitro-3-carboxybenzisoxazole are general. To the extent that the lines are not perfectly parallel, the solvent sensitivity increases with more electron-withdrawing substituents and decreasing carboxylate basicity. The Hammet ρ values for the substituent effects are essentially similar in most solvents except those producing extreme rates, e.g., water at one end of the scale and dimethylacetamide and hexamethylphosphoramide at the other end of the scale. These results, taken with the measurements of activation entropies, indicates that little change in transition-state occurs.[1]

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(43) In addition, the rate data plotted against the H parameter did not fall on a single line, and the three lines drawn by the authors grouped dissimilar solvents. For example, water, carbon tetrachloride, and formamide fall on a single line, while amides are found on two separate lines, as are solvents with hydroxyl groups.[1]

Table II. Comparison of the Solvent Sensitivities of Decarboxylation and E2 Elimination Reactions

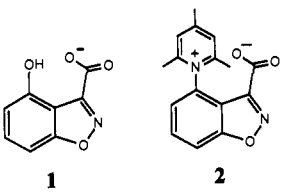
	decarboxylation			E2	
solvent	$TMGH^+$, log k^a	**1**, log k^b	**2**, log k^c	acetate, log k^d	Et_3N, log k^e
water	−5.13	−5.89	−6	−5.57	−0.09
formamide	−3.13				−0.43
benzene	−2.32			1.52	
benzonitrile	0.40			1.26	
acetonitrile	0.46	−6.42	0.36	0.18	0.25
dimethyl sulfoxide	1.00	−4.44			0.63
acetone	1.38		1.1	1.52	
N,*N*-dimethylformamide	1.56	−5.05	1.4		0.45
N,*N*-dimethylacetamide	2.20	−4.92			
hexamethylphosphoramide	2.80				

[a] Rates for the decarboxylation of 6-nitro-3-carboxybenzisoxazole at 30 °C from ref 1; reaction initiated with TMG. [b] Rates for the decarboxylation of the hydroxy-substituted carboxybenzisoxazole **1** at 30 °C, except for water at 50 °C, from ref 2. [c] Rates for the decarboxylation of the pyridinium-substituted carboxybenzisoxazole **2** at 30 °C from ref 4. [d] Rates for the E2 reaction of unsubstituted benzisoxazole with acetate ion at 30 °C from ref 2. [e] Rates for the E2 reaction of 5-nitrobenzisoxazole with triethylamine at 30 °C from ref 2.

separating the relevant ion pairs. Solvents that are dipolar and able to participate in hydrogen-bonding interactions are likely to be the best for solvating the reactant carboxylate and its $TMGH^+$ counterion. We will refer to solvents without these solvating abilities as "weak" solvents. Clearly, benzene and carbon tetrachloride are the weakest solvents in the set followed by diethyl ether and dioxane. This order can be confirmed by creating a coarse scale of solvating power by summing the π_1^*, α_1, and β_1 parameters for each solvent; this approach ranks the solvents in the same order that one would expect from a general chemical understanding of solvent properties and is similar to the ranking of solvents by the E_T parameter.[19] Removing the above four solvents from the solvent set and reregressing the data against all five parameters yielded eq B5 in Table III. Next we removed dichloromethane, chloroform, and tetrahydrofuran. The remaining 13 solvents were regressed against the five parameters to give eq C5 in Table III. We continued by removing benzonitrile to obtain eq D4 for 12 solvents and then removed acetone to obtain eq E4 for 11 solvents. The latter two equations only have four parameters because there are no solvents with nonzero δ values in these solvent sets.

As the weakest solvents are removed from the original 20-solvent data set, the *s*- and *h*-coefficients (for parameters π_1^* and $(\delta_H^2)_1$, respectively) drop in absolute value to near zero. Therefore, these two terms can be dropped from the regression. The correction factor δ is dropped with π_1^*.[44] Therefore, we reregressed the progressively smaller solvent sets against α_1 and β_1 alone to obtain eqs A2–E2 in Table III. The entire 20-solvent data set is rather poorly fit by just two parameters ($R = 0.906$), but the fit improves dramatically after the four least polar solvents are removed ($R = 0.978$). Subsequent regressions with fewer solvents change very little. Solvent hydrogen-bond-donating power is indicated as a strong rate–retarding effect by the large negative *a*-coefficients, while hydrogen-bond basicity is indicated as an accelerating factor. The two-parameter equations fit the reduced data sets as well as the five-parameter equation fits the entire data set. Rates calculated according to eq C2 are plotted against the experimental rates in Figure 1b. This plot includes the rates calculated for the 13 solvents used to derive the regression line, as well as the rates calculated for the remaining nine solvents in Table I.

We have also examined two other related data sets. The pyridinium-substituted compound **2** decarboxylates at similar rates to 6-nitrobenzisoxazole-3-carboxylate and exhibits similar solvent effects. Rate data for this compound are in Table II. Although data are only available for four solvents, the rates span over 6 orders of magnitude, and we find that they correlate extremely well with the solvent α_1 parameter ($R > 0.999$):

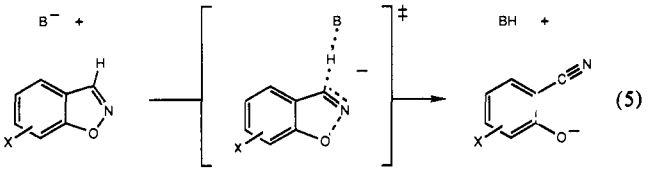


1 **2**

$$\log k = 1.48 - 5.69\alpha_1 \quad (4)$$

This correlation is shown in Figure 2, plotting the decarboxylation rates against solvent α_1 values.[45] Eliminating water from the data set and regressing the remaining three solvents against α_1 gives the same equation with $R = 0.991$. The coefficient for α_1 in eq 4 is essentially the same as the coefficients for α_1 in eqs B2–E2 (Table III).

The E2 ring-opening reaction between a base (B) and benzisoxazole yields the same cyanophenolate product as the decarboxylation reaction in eq 1 and involves a similar transition state:



(5)

Rates for two such E2 reactions are given in Table III. When the base is acetate, the E2 reaction is very solvent-sensitive, with rates that increase by 7 orders of magnitude from reaction in water to reaction in benzene or benzonitrile.[2] By contrast, the E2 reaction with triethylamine is virtually solvent-insensitive,[2] a point to which we will return in the discussion. We find that the limited rate data available (five solvents) for the solvent-sensitive reaction of unsubstituted benzisoxazole with acetate correlate with the hydrogen-bond acidity parameter α_1 according to eq 6 with $R = 0.995$:

$$\log k = 1.53 - 6.07\alpha_1 \quad (6)$$

This equation is quite similar to eq 4 for the decarboxylation of compound **2**, and the coefficient for α_1 is similar to the coefficients for α_1 in eqs B2–E2 (Table III).

Discussion

Mechanisms for Solvent Sensitivity. Although the interpretation of statistical correlations must always be done with care, the Kamlet–Abraham–Taft technique appears to be one of the most effective methods currently available for dissecting solvent-induced effects on rates and equilibria into individual components. Three potential mechanisms for solvent sensitivity have been

(44) The δ parameter disappears in any case because there are no solvents with nonzero δ values in the reduced solvent sets D and E.

(45) The mechanistic significance of this correlation depends on the accuracy with which the α_1 parameter estimates the hydrogen-bond donor strength of weak donors like acetonitrile. In addition to the original studies by Taft and co-workers, where several model systems were examined to establish the α_1 scale and the precision of this parameter for acetonitrile was as good as for the other solvents,[24,30] we note that Coetzee and Sharp have shown that the C–H stretching frequency of acetonitrile is shifted by dissolved anions.[46] The occurrence of hydrogen bonding between oxygen bases and a variety of carbon acids, including nitriles, has been noted by Pedireddi and Desiraju in a study of C–H···O hydrogen-bond distances in a survey of 551 crystal structures.[47] Murray and Politzer have found that the hydrogen-bond acidities measured by the α_1 parameter correlate with surface electrostatic potentials,[48] and Lorand has measured equilibrium constants for the complexation of a variety of carbon acids with various bases by NMR.[49] Additional relevant studies are in refs 50 and 51.

Table III. Five-Parameter, Four-Parameter, and Two-Parameter Regression Equations on Solvent Sets that Progressively Exclude Solvents where Ion Pairing Is Prevalent

		$\log k$ = constant + $s\pi_1^* + d\delta + a\alpha_1 + b\beta_1 + h(\delta_H^2)_1$							
eq no.	n	constant	s	d	a	b	h	R^h	σ^h
A5	20[a]	−2.97 (±0.52)[f]	5.45 (±0.89)[g]	−1.46 (±0.45)	−3.03 (±0.65)	1.80 (±0.66)	−1.06 (±0.25)	0.976	0.58
B5	16[b]	−1.28 (±1.18)	2.94 (±1.87)	−1.16 (±0.63)	−4.38 (±0.99)	1.73 (±0.62)	−0.57 (±0.38)	0.985	0.53
C5	13[c]	1.31 (±2.87)	−0.57 (±5.03)	−0.92 (±0.67)	−5.86 (±2.55)	1.54 (±1.04)	−0.03 (±0.98)	0.988	0.53
D4	12[d]	1.31 (±2.87)	−0.57 (±5.03)	*i*	−5.86 (±2.55)	1.54 (±1.04)	−0.03 (±0.98)	0.988	0.53
E4	11[e]	0.15 (±3.66)	1.14 (±6.11)	*i*	−5.01 (±3.08)	1.48 (±1.10)	−0.33 (±1.16)	0.988	0.55

		$\log k$ = constant + $a\alpha_1 + b\beta_1$				
eq no.	n	constant	a	b	R	σ
A2	20[a]	−1.41 (±0.46)	−4.74 (±0.63)	3.92 (±0.80)	0.906	1.02
B2	16[b]	0.01 (±0.33)	−5.63 (±0.37)	2.42 (±0.50)	0.978	0.55
C2	13[c]	0.36 (±0.48)	−5.73 (±0.36)	2.02 (±0.70)	0.983	0.53
D2	12[d]	0.76 (±0.50)	−5.90 (±0.34)	1.57 (±0.69)	0.987	0.48
E2	11[e]	0.60 (±0.56)	−5.83 (±0.36)	1.73 (±0.74)	0.987	0.49

[a] The solvent set in Table I. n gives the number of solvents. [b] The solvent set in Table I minus carbon tetrachloride, benzene, diethyl ether, and dioxane. [c] Dichloromethane, chloroform, and tetrahydrofuran have also been removed from the solvent set. [d] Benzonitrile has also been removed from the solvent set. [e] Acetone has also been removed from the solvent set. [f] The numbers in parentheses are the standard errors of the coefficients. [g] The number in the table is the coefficient. [h] R is the multiple correlation coefficient, and σ is the root mean square error. [i] All δ values for the remaining solvents are 0, so there is no $d\delta$-term.

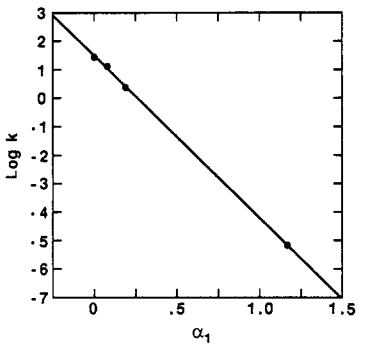


Figure 2. Rates of decarboxylation of compound **2** in water, acetonitrile, acetone, and *N*,*N*-dimethylformamide (in order of increasing rate) plotted against the solvent hydrogen-bond acidity parameter α_1. The line shown is the simple linear regression line given in the text as eq 4.

identified in previous studies: hydrogen-bond donation by solvent, ion-pairing effects, and transition-state stabilization by dispersion interactions.[1,2,17,20] In the discussion below, we examine each of these mechanisms in turn, in light of our regression results and additional experimental data that are available.

The importance of hydrogen-bond donation by solvent has been recognized since the first studies on the solvent dependence of this reaction, and its effect is logically ascribed to stabilization of the reactant carboxylate.[1,2] In addition to the general observation that the reaction is slowest in protic solvents, studies on compound **1** demonstrated the profound effect of hydrogen bonding.[2] This compound decarboxylates slowly in all solvents; the intramolecular hydrogen bond donated by the 4-hydroxy substituent renders the surrounding medium practically irrelevant. Electronic effects were eliminated as a factor in this result by comparisons with 6-hydroxy- and 6-methoxy-substituted benzisoxazole-3-carboxylates, which both exhibit large solvent effects.

Hydrogen-bond donation by solvents is specifically addressed by the a α_1-term in the regression analysis. In the two-parameter regression equations, B2–E2, the large negative a-coefficients of −5.6 to −5.9 identify solvent hydrogen-bond acidity as a very influential solvent property that retards the rate. Studies on compound **2** also indicate a large effect of hydrogen bonding. This pyridinium-substituted compound decarboxylates at rates that are quite sensitive to solvent, with the slowest rate in water, and we find that the limited rate data available correlate quite well with solvent hydrogen-bond acidity (eq 4 and Figure 2).[45] The a-coefficient of −5.69 in this correlation is similar to the values in eqs B2–E2. The E2 reaction of benzisoxazole with acetate represents another related reaction that is solvent-sensitive, and the rates available appear to depend on solvent hydrogen-bond acidity (eq 6). The a-coefficient in this case is −6.07.

These regression results allow us to assign a magnitude to the hydrogen-bonding effect. Taking −5.9 as a representative value, we find that for a range of α_1 values from 0 for aprotic solvents to 1.17 for water, the solvent hydrogen-bond donation effect can account for ca. 7 orders of magnitude of rate variation. The total rate variation among the solvents in the decarboxylation of 6-nitrobenzisoxazole-3-carboxylate is 7.9 log units (Table I), while the ranges for the decarboxylation of **2** and the E2 reaction involving acetate (Table II) are 7.4 and 7.1 log units, respectively. Hydrogen-bond donation by solvent is clearly a very large factor in the observed solvent sensitivities.

Previous studies have also indicated that ion pairing can significantly influence the rates of the decarboxylation, at least in some solvents.[1,17,20] This is a complicating factor in the interpretation and correlation of the rate data for the decarboxylation of 6-nitrobenzisoxazole-3-carboxylate in the presence of $TMGH^+$. Ion pairs undoubtedly exist in the least polar aprotic solvents, as originally indicated by Kemp and supported by the studies of Smid.[1,17,20] Potential ion-pairing equilibria are given in eq 7. In this equation, $(TMGH^+\cdots{}^-O_2CR)_{solv}$ represents tight

$$(TMGH^+\cdots{}^-O_2CR)_{solv} \rightleftharpoons (TMGH^+)_{solv}({}^-O_2CR)_{solv} \rightleftharpoons (TMGH^+)_{solv} + ({}^-O_2CR)_{solv} \quad (7)$$

hydrogen-bonded ion pairs, $(TMGH^+)_{solv}({}^-O_2CR)_{solv}$ represents solvent-separated ion pairs, and $(TMGH^+)_{solv} + ({}^-O_2CR)_{solv}$ represents free ions. Previous studies have not explicitly noted the possibility of hydrogen-bonded ion pairs between the carboxylate and $TMGH^+$. This interaction, however, provides a mechanism by which ion pairing should have a large effect on the decarboxylation rates, given the profound rate-retarding effects of hydrogen bonding.

There is no specific "ion-pairing" term in the regression equation, but it is expected that particular solvent properties will influence the positions of the ion-pairing equilibria. In addition, we explored ion-pairing effects by selectively reducing the solvent sets. Three aspects of the regression analysis are consistent with ion-pairing effects: the positive b-coefficient, the trends in the $(s\pi_1^* + d\delta)$-term as solvent sets were reduced, and the trends in the constant as solvent sets were reduced. A fourth aspect of the analysis, the h-coefficient for cavity effects, may also be related.

The positive b-coefficient for β_1 in both the five- and 2-parameter regression equations indicates that the reaction is

promoted by basic solvents, an effect that can be rationalized by the ability of basic solvents to solvate the $TMGH^+$ counterion and modify its interactions with the carboxylate.[52] Thus, basic solvents may compete with the carboxylate for the hydrogen-bond acidic cation, separating the ions and promoting the reaction. This interpretation seems reasonable since studies by Kolthoff indicate that the dissociation of hydrogen-bonded ion pairs is dependent on solvent basicity, and $TMGH^+$ is a hydrogen-bond acidic species.[53,54] In addition, experiments by Kolthoff have established that carboxylates can form complexes with hydrogen-bond donor species even in strongly basic dipolar solvents such as dimethyl sulfoxide and dimethylformamide.[55,56] Carboxylate anions are strongly destablized in these solvents and should be particularly avid in seeking out stabilizing interactions: the only such interactions available in the aprotic solvents are with $TMGH^+$.

However, the significance of this mechanism across the entire solvent set, including the dipolar aprotic solvents, is not entirely clear. The two-parameter regression model attributes the rate variations among dipolar aprotic solvents to incomplete dissociation of ion pairs which is modulated by solvent basicity. This interpretation requires that ion pairs in fact exist in these quite basic dipolar solvents. The positions of the equilibria in eq 7 in various dipolar aprotic solvents are not known in detail, but we can make comparisons with other measured ion-pairing equilibria. Kohlthoff has determined a dissociation constant of 1.2×10^{-5} M in acetonitrile for the hydrogen-bonded ion pair trimethylammonium 3,5-dinitrobenzoate,[54] and dissociation constants for tetramethylguanidinium benzoate and tetramethylguanidinium 3,5-dinitrobenzoate ion pairs in acetonitrile are reported to be 3.6×10^{-4} and 1.4×10^{-3} M, respectively.[57] Assuming similar dissociation constants for tetramethylguanidinium benzisoxazole-3-carboxylates, estimates of 99, 80, or 50%, respectively, can be calculated for the fraction of the reactant carboxylate that is ion paired in acetonitrile under Kemp's typical reaction conditions (total benzisoxazole-3-carboxylate concentration of 0.0002 M and a total $TMGH^+$ concentration of 0.0014 M).[58] All these dissociation constants imply that there will be a significant amount of ion pairing in acetonitrile. We have not found literature data for the extent of ion pairing of tetramethylguanidinium salts in more basic dipolar aprotic solvents, such as dimethyl sulfoxide, dimethylformamide, and hexamethylphosphoramide, but it will certainly decrease considerably relative to acetonitrile.

While hydrogen bonding is one possible mechanism by which $TMGH^+$ influences decarboxylation rates, it is apparent from other data that charge–charge interactions in contact ion pairs involving alkali metal cations can also stabilize the carboxylate and decrease reaction rates. Smid's studies with crown-complexed alkali metal cations in benzene support the idea that tight ion pairs decarboxylate with rates that are quite sensitive to interionic distances.[17] In acetonitrile, Kemp found that added alkali metal cations significantly decreased decarboxylation rates.[1] In dimethyl sulfoxide, added Li^+, Na^+, and K^+ had little effect on the decarboxylation rate, however, indicating that tight ion pairing under these conditions is neglible.[1] The presence of a positively-charged pyridinium ion near the carboxylate in **2** did not retard the reaction significantly in any solvent or alter the pattern of solvent sensitivity (Table II).[4] This charge may not be as close to the carboxylate as those of contacting alkali metal cations in acetonitrile. In addition, unlike $TMGH^+$, the pyridinium cation cannot donate a hydrogen bond. These results suggest that the solvent-separated ion pair in eq 7 may decarboxylate at a much faster rate than the tight ion pair, possibly as fast as a free ion.

Solvent polarity is also expected to influence ion-pairing equilibria. Among the aprotic solvents in the entire data set, there is a clear trend of increasing rate with increasing solvent polarity, and this is confirmed in eq A5 by the net positive contribution of the $(s\pi_1^* + d\delta)$-term. However, as the least polar aprotic solvents are removed from the data set, the s-coefficient drops toward zero and becomes statistically insignificant (see eqs A5–E4 in Table III). Reducing the solvent set in this manner significantly simplified the regression results, reducing a five-parameter correlation to a two-parameter correlation. In Figure 1b, it is particularly evident how the rates in the least polar aprotic solvents (i.e., carbon tetrachloride, benzene, dioxane, and diethyl ether) deviate from those in the rest of the solvent set. Since solvents that most disfavor the dissociation of ion pairs were eliminated in the course of this analysis, this result suggests that solvent dipolarity and polarizability may have a greater influence on the ion-pairing equilibria than on the decarboxylation step itself.

Trends in the regression constant (Table III) as the solvent sets are reduced are also consistent with the occurrence of ion pairing in the reaction system. The value of the constant indicates the rate of the reaction in a solvent where all the explanatory parameters have values near zero, such as in an alkane or, hypothetically, in the gas phase. The negative value of the constant in eq A5 indicates that the reaction should be quite slow in an alkane, which is exactly as expected since ion pairing should be very tight in a nonpolar microenvironment. As the solvent sets are reduced, the resulting regression equations become increasingly ignorant of the retarding effects of ion pairing in aprotic solvents of low polarity. The constants increase substantially, from –2.97 in eq A5 to 1.31 in eq D4 and from –1.41 in eq A2 to 0.76 in eq D2, indicating that the expected decarboxylation rate would be substantial in a hypothetical medium with little dipolarity, hydrogen-bond acidity, or hydrogen-bond basicity, so long as the carboxylate anion could be dissociated from its counterion. Smid's work on the accelerating effects of crown ethers in benzene and his studies of the apparent free-ion rate constants in ethers are consistent with this view as they suggest that the rate at which the free ion decarboxylates in nonpolar solvents can approach or even exceed the rates achieved in the most dipolar aprotic media.[17,20]

The negative h-coefficient for $(\delta_H^2)_1$ in eq A5 also provides possible support of the ion-pairing model. It suggests that the cost of expanding the cavity that contains the reactant can retard the reaction in cohesive solvents. This result is consistent with the expectation that the solvent shell must expand as ion pairs dissociate. As the solvent sets were reduced by eliminating solvents that most disfavor ion-pair dissociation, the h-coefficient dropped to zero and became statistically insignificant, suggesting that cavity effects are more influential on the ion-pairing equilibria than on the decarboxylation step. However, some caution is

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(52) Kemp had previously observed that reaction rates among ethers increased with basicity, although he considered it unlikely that this involved interactions with the tetramethylguanidinium counterion.[1] We find a correlation with basicity across a broad range of solvents, and it persists even in reduced solvent sets that do not include the ethers.
(53) Kolthoff, I. M.; Chantooni, M. K. *J. Am. Chem. Soc.* **1969**, *91*, 2875–2883.
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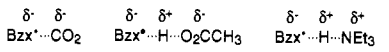
(56) These stability constants were not large for this neutral hydrogen-bond donor, but larger constants might be expected with a cationic donor such as $TMGH^+$ where charge–charge interactions also come into play.
(57) Chantooni, M. K.; Kolthoff, I. M. *J. Phys. Chem.* **1976**, *80*, 1307–1310.
(58) $TMGH^+$ cation was generated from *p*-toluenesulfonic acid added to the reaction medium to suppress spontaneous decarboxylation in organic solvents prior to initiating the reactions with excess TMG.[1,3]

warranted in the interpretation of these particular regression results.[59] Although the error in the h-coefficient is low in eq A5, the $(\delta_H^2)_1$ parameters in the 20-solvent set are cross-correlated with both the π_1^* and α_1 parameters, as determined by a correlation matrix (cross correlations: α_1 and $(\delta_H^2)_1$, 0.777; π_1^*, and $(\delta_H^2)_1$, 0.556).[60]

Transition-state stabilization is the third factor that has been proposed to influence the rates of decarboxylation. The evidence for transition-state stabilization derives mainly from studies of solvent-extraction catalysis in which cation-dependent rate accelerations of 10^2–10^4 were observed in two-phase water/benzonitrile systems relative to the rate in water.[2] The observation of such catalysis indicates that the transition state must be stabilized in the organic phase relative to water. Kemp has argued that dispersion interactions between the transition state and the organic solvent must be the source of this stabilization, given the charge-delocalized polarizable nature of the transition state. If the rate range observed in dipolar aprotic solvents (i.e., from 10 s^{-1} in dimethyl sulfoxide to ca. 700 s^{-1} in hexamethylphosphoramide[1]) is not due to variations in ion pairing, then some other factor must be operating, and transition-state stabilization via dispersion interactions represents a possible accelerating influence.

Regression analyses on solvent-dependent rates do not separate transition-state effects from ground-state effects and can only identify particular interactions that contribute to a net increase or decrease in the activation energy. Our regression analysis did not identify any specific factor indicating a role for dispersive interactions. The $(s\pi_1^* + d\delta)$-term dropped out of the regression equations once the solvents with the least ability to separate ion pairs were removed from the data set, even though the solvents remaining still spanned a large range in polarity (i.e., as indicated by π_1^* values and properties such as the dielectric constant). This result suggests that any influence of solvent dipolarity/polarizability on the transition state may be paralleled by a similar influence on the ground state, such that there is no net effect on the activation energy. This conclusion must be considered tentative, however, since the regression method using the $(s\pi_1^* + d\delta)$-term is not particularly effective in distinguishing dispersion and dipolar interactions. The solvent extraction results are clear in indicating that the transition state is stabilized in the organic solvent relative to in water, but it is difficult to determine from these studies alone whether or not the same interactions stabilize the ground state to a similar degree on transfer from water to the organic solvent, since ground-state destabilization by reduction in hydrogen bonding in the organic solvent is occurring simultaneously.[61]

Experimental studies of the E2 elimination reaction of benzisoxazoles in eq 5 may shed some light on the importance of dispersion interactions, since it involves a similar transition state and there is no possibility of hydrogen-bonded ion pairing. The solvent insensitivity of the E2 reaction involving triethylamine suggests that dispersive interactions are not a significant effect when contrasted with the large solvent sensitivities of the E2 reaction with acetate and the decarboxylation reaction. All these reactions delocalize charge into the benzisoxazole structure in the transition state, likely rendering it more polarizable (eqs 1 and 5). The carboxylate in the decarboxylation reaction loses charge and becomes less polarizable at the same time. Using Bzx* to represents the benzisoxazole structure as it proceeds toward the cyanophenolate ion, the transition-state structures for these three reactions can be illustrated by structures **3**, **4**, and **5**. If transition-state stabilization by dispersive interactions is



3 **4** **5**

occuring, then those interactions should act on the Bzx* as it becomes more polarizable and all three reactions should be solvent-sensitive. However, only those reactions involving carboxylate ions, as in **3** and **4**, are solvent-sensitive. Rates vary by only a factor of 5 from water to dimethyl sulfoxide in the reaction with triethylamine. These results show that large solvent effects reflect the activity of the carboxylate functionality in the test solvent rather than solvent interactions with the polarizable benzisoxazole. Consequently, the contribution of dispersion interactions is likely to be relatively small compared to the much larger hydrogen-bonding effects already noted.

Our analysis suggests additional experiments that might be conducted to clarify the role of $TMGH^+$ and ion pairing in the full range of solvents. Since significant variations in decarboxylation rates among dipolar aprotic solvents where $\alpha_1 = 0$ have only been demonstrated in solutions containing $TMGH^+$ (see Table III), it would be useful to conduct further studies on related systems that do not include $TMGH^+$, i.e., the decarboxylation of the pyridinium-substituted carboxybenzisoxazole (compound **2**) and the E2 reaction with acetate as the base. These have not been studied in a sufficient range of solvents (see Table III) to establish if rate variations among the dipolar aprotic solvents are general for these types of reactions or if they are unique to those containing a hydrogen-bond-donating cation. Ion pairing issues might also be further explored by varying the total $TMGH^+$ concentration in a variety of the more basic dipolar aprotic solvents and observing the effect on decarboxylation rates. This experiment was only done for one solvent in previous studies: changing the $TMGH^+$ concentration from 0.0003 to 0.03 M did not affect the rate in acetonitrile, where the dissociation constants cited as models above indicate that there is a significant degree of ion pairing in this concentration range.[66] Similar experiments in more basic dipolar aprotic solvents such as dimethylformamide and hexamethylphosphoramide might be informative.

To summarize, our analysis identifies hydrogen-bond donation by solvent as the largest single factor influencing the decarboxylation rates, accounting for perhaps 7 orders of magnitude in rate variation. Ion-pairing interactions also influence the rate in at least a subset of the aprotic solvents, and this is likely due to

(59) The $(\delta_H^2)_1$ parameter, in combination with the other four contributes to the predictive accuracy of eq A5. The fit drops from $R = 0.976$ to 0.947 if the full data set is only regressed against the other four parameters.

(60) The cross correlation between α_1 and $(\delta_H^2)_1$ arises because the solvents that have the highest cohesive energy densities are those that are capable of forming hydrogen-bonded networks, i.e., hydrogen-bond acids. Cross correlations between parameters were reduced by selectively removing the solvents making the largest contributions to those cross correlations. Removing water, formamide, methanol, diethyl ether, and carbon tetrachloride reduced the cross correlation between α_1 and $(\delta_H^2)_1$ to 0.294 and the cross correlation between π_1^* and $(\delta_H^2)_1$ to 0.385. Regressing the rates in the remaining 15 solvents against all five parameters resulted in an equation that is similar to eq A5, with $h = -1.61 \pm 0.78$.

(61) One conclusion derived from the solvent-extraction studies was that the affinity between water and the substrate in the organic phase is less than the corresponding affinity in the aqueous phase.[2] We attribute this reduced affinity to the differing hydrogen-bond-donor strengths of water in the two phases. Abraham has noted that the hydrogen-bond-donor strength of monomeric water is less than that of solvent water, and Carr has discussed the observation that self-associating species (i.e., those that are both hydrogen-bond acids and bases) are less hydrogen-bond acidic as monomers than as solvents.[62,63] Abboud and co-workers showed that alcohol dimers are stronger hydrogen-bond acids in the bulk liquid phase than are monomeric alcohols.[64,65] The number and strength of hydrogen bonds to the carboxylate may be less in hydrated benzonitrile than in water, such that the hydrated reactant in the wet organic phase is more reactive than in bulk water.

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(66) Since acetonitrile is itself a hydrogen-bond acid, it can stabilize the carboxylate if the $TMGH^+$ does not. This is a potentially complicating factor in the interpretation of these results.

the rate-retarding effect of tight hydrogen-bonded ion pairs with $TMGH^+$. Solvent basicity may modulate the extent of hydrogen-bonded ion pairing. Rate variations among the most basic dipolar aprotic solvents may be due either to variations in the extent of ion pairing, as suggested by Smid and the dependence on solvent basicity in our regression analysis, or to transition-state stabilization effects involving dispersive interactions, as postulated by Kemp in his original studies. We have identified further experiments that might clarify the role of ion pairing in these solvents. If tight ion-pairing effects were operative across the entire range of aprotic solvents, then this could unify all the rate variations in terms of hydrogen-bonding effects. Exploration of the role of dispersive interactions, involving matching of polarizable portions of the solvent with polarizable portions of the transition state, may require rigid solvent environments, as originally proposed by Kemp.[2] Recent studies by Dougherty have exploited cyclophane hosts as structured microenvironments for exploring the relative roles of electrostatic and dispersion interactions in catalysis of alkylation and dealkylation reactions.[67]

Antibody Catalysis. The monoclonal antibody 21D8 was elicited in response to a 1,5-naphthalenedisulfonate hapten and efficiently catalyzes the decarboxylation of 5-nitrobenzisoxazole-3-carboxylate.[18] In contrast to typical enzymes, where a variety of accelerating factors operate simultaneously (such as specific catalytic groups and proximity effects), 21D8 provides a simple model system for studying the solvation properties of a protein binding pocket. It is generally believed that desolvation of substrate and transition state by the active site of an enzyme can play a significant role in the rate accelerations achieved by these catalysts.[29]

From the analysis of solvent effects above, it is clear that acceleration of this decarboxylation reaction must involve a reduction in hydrogen-bond donation to the substrate when it is transferred from water into the antibody binding site.[68] However, the catalytic rate acceleration of 10^4 relative to that of the reaction in water is still considerably less than the rate accelerations observed in some of the solvent environments considered above. These observations suggest the testable hypothesis that a hydrogen-bond donor is present in the active site near the carboxylate group of the substrate. This donor is apparently less effective than an aqueous solvent shell at retarding the decarboxylation rate but still prevents the reaction from proceeding as rapidly as might be expected if the carboxylate were completely free of hydrogen bonds. Because antibody 21D8 was isolated after immunizations with a negatively-charged hapten, we consider it quite likely that the binding site contains a positively-charged amino acid residue, such as a protonated lysine or a protonated arginine.[18,69] In order to catalyze the decarboxylation reaction, a positive charge is probably necessary to attract the negatively-charged carboxylate from water into the organic binding pocket and provide the binding energy necessary to overcome the loss of solvation energy when the carboxylate is transferred out of water. If such a cation is present in the active site, it is also likely to be the postulated hydrogen-bond donor.

However, it is worth noting that such an active site group is not as effective at donating a hydrogen bond as the phenol in compound **1**, where the hydrogen bond is intramolecular. The postulated hydrogen bond in the binding site may be weaker than the intramolecular hydrogen bond, or there may be an equilibrium between hydrogen-bonded and non-hydrogen-bonded substrates. Factors that might influence the strength of a hydrogen bond include the type of hydrogen-bond donor, the length of the hydrogen bond, which may be constrained by the binding site geometry, and the polarity of the microenvironment around the hydrogen bond. Microenvironment polarity could also influence the position of an equilibrium between hydrogen-bonded and non-hydrogen-bonded substrates. It is interesting to consider that of 1200 hapten-binding antibodies screened, only 2% had significant catalytic activity.[18] It may be that in most of these antibodies, the substrate bound in the active site forms a hydrogen bond to a cationic residue that is as effective at retarding decarboxylation as the intramolecular hydrogen bond in compound **1**.

Because catalytic antibodies provide a discrete rigid microenvironment for chemical reaction to occur and because their structures are amenable to alteration by a wide range of protein-engineering techniques, the isolation of even a moderately successful catalyst can provide the starting point for the development of better catalysts if the factors governing catalytic efficiency are understood. Although the structure of the antibody 21D8 is not yet available, our analysis of the medium effects above allows us to identify the properties of the active site that are most relevant to the catalyzed decarboxylation reaction. Efforts to improve this antibody's catalytic efficiency should focus on reducing hydrogen-bond donation in the active site while maintaining adequate substrate binding. For example, a lysine residue, if present at the active site, could be N-methylated to reduce hydrogen bonding while retaining the positive charge. Alternatively, protein engineering might be able to reorient or reposition the charge to lengthen the hydrogen bond or to prevent hydrogen-bonding contact altogether, while still allowing a charge–charge interaction.

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(67) McCurdy, A.; Jimenez, L.; Stauffer, D. A.; Dougherty, D. A. *J. Am. Chem. Soc.* **1992**, *114*, 10314–10321.

(68) This conclusion is consistent with the results obtained from experiments using fluorophores that bind to the catalytic site.[18] These studies demonstrated that the aqueous solvent shell is stripped from the substrate when it is bound and that the bound substrate is not accessible to small probe ions or molecules.

(69) Catalytic antibodies were not successfully isolated from immunizations with neutral haptens.