Biomimetic Catalysis of S_N2 Reactions through Cation-π Interactions. The Role of Polarizability in Catalysis

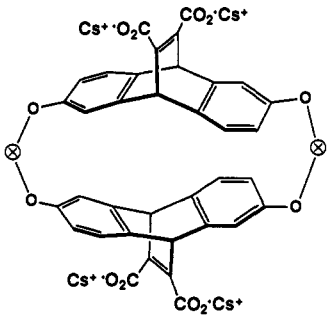
Alison McCurdy, Leslie Jimenez,[†] David A. Stauffer,[‡] and Dennis A. Dougherty*

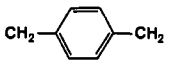
Contribution No. 8648 from the Arnold and Mabel Beckman Laboratories of Chemical Synthesis, California Institute of Technology, Pasadena, California 91125. Received June 5, 1992

Abstract: Cyclophane hosts **1** and **2** have been shown to be effective catalysts for both the alkylation of quinoline structures to produce quinolinium salts and the dealkylation of sulfonium salts to produce sulfides. Thus, reactions that develop positive charge in the transition state and reactions that destroy positive charge are accelerated. The former observation is not surprising, given the well-documented ability of these hosts to bind cations through the cation-π interaction. The catalysis of the dealkylation reactions, however, along with several other observations, suggests that some other factor is involved in the catalysis. It is proposed that the high polarizability of the transition states is well matched to the very polarizable hosts and that this contributes to the catalysis.

In recent years, it has become apparent that aromatic rings can serve as relatively polar groups, especially in a biological context. The edge of a benzene ring is positively charged, and the face is negatively charged. These considerations explain a number of phenomena, including the large quadrupole moment of benzene itself,[1] the T-shaped benzene-benzene interaction seen in the gas phase and in the crystal,[2] the recently observed hydrogen-bonding complex between benzene and water,[3] and the orientation of aromatic-aromatic interactions in protein structures.[4] The "edge-to-face" interaction has also been invoked in several synthetic host-guest complexes.[5]

Our own work has emphasized the stabilizing interaction between a positive charge and the electron-rich face of an aromatic ring.[6,7] This "cation-π" interaction presumably involves a number of fundamental forces, such as charge-dipole, charge-quadrupole, charge-induced dipole, and London dispersion terms. In our host systems, exemplified by structures **1** and **2**, the effect is especially



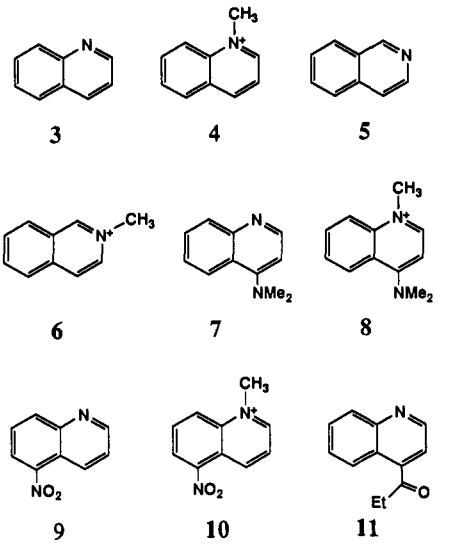
1: ⊗ = 

2: ⊗ = 

pronounced when the cation guest is an "onium" compound, such as a tetraalkylammonium or an alkylated pyridinium structure. In these instances, a significant hydrophobic effect augments the cation-π interaction, producing very high binding affinities for relatively water-soluble guests. We have proposed that such interactions are important in biological recognition of the neurotransmitter acetylcholine,[8] and this proposal has received considerable recent support.[9]

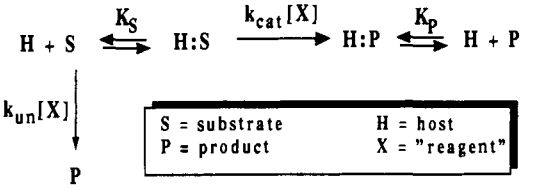
The present work assesses the ability of hosts such as **1** and **2** to serve as catalysts for reactions that involve positively charged transition states. In a previous preliminary report,[10] we showed that the reaction of quinoline and its derivatives with methyl iodide

Chart I



3 **4** **5** **6** **7** **8** **9** **10** **11**

Scheme I



to form quinolinium compounds—a Menschutkin reaction[11] —was catalyzed by **1** and **2**.[12] Analysis of the results suggested, among

[†] Present address: Department of Chemistry, Rutgers University, Piscataway, NJ 08855.
[‡] Present address: Center for Molecular Recognition, Columbia University, New York, NY 10032.

(1) Dennis, G. R.; Ritchie, G. L. D. *J. Phys. Chem.* **1991**, *95*, 656-660. Laidig, K. E.; *Chem. Phys. Lett.* **1991**, *185*, 483-489.
(2) Cox, E. G.; Cruickshank, D. W. J.; Smith, J. A. S. *Proc. R. Soc. London* **1958**, *247*, 1. Wykoff, R. W. G. *Crystal Structures, The Structure of Benzene Derivatives*; Interscience: New York, 1969; Vol. 6. Burley, S. K.; Petsko, G. A. *J. Am. Chem. Soc.* **1986**, *108*, 7995-8001.
(3) Suzuki, S.; Green, P. G.; Bumgarner, R. E.; Dasgupta, S.; Goddard, W. A., III; Blake, G. A. *Science* **1992**, *257*, 942-945.
(4) Burley, S. K.; Petsko, G. A. *Adv. Protein Chem.* **1988**, *39*, 125-189. Burley, S. K.; Petsko, G. A. *FEBS Lett.* **1986**, *203*, 139-143.
(5) See, for example: Muehldorf, A. V.; Van Engen, D.; Warner, J. C.; Hamilton, A. D. *J. Am. Chem. Soc.* **1988**, *110*, 6561-6562. Cochran, J. E.; Parrott, T. J.; Whitlock, B. J.; Whitlock, H. W. *J. Am. Chem. Soc.* **1992**, *114*, 2269-2270.
(6) (a) Shepodd, T. J.; Petti, M. A.; Dougherty, D. A. *J. Am. Chem. Soc.* **1986**, *108*, 6085. (b) Shepodd, T. J.; Petti, M. A.; Dougherty, D. A. *J. Am. Chem. Soc.* **1988**, *110*, 1983. (c) Petti, M. A.; Shepodd, T. J.; Barrans, R. E., Jr.; Dougherty, D. A. *J. Am. Chem. Soc.* **1988**, *110*, 6825. (d) Stauffer, D. A.; Dougherty, D. A. *Tetrahedron Lett.* **1988**, *29*, 6039. (e) Stauffer, D. A., Barrans, R. E., Jr.; Dougherty, D. A. *J. Org. Chem.* **1990**, *55*, 2762-2767.

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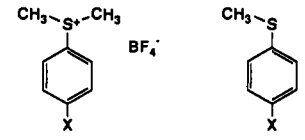
Chart II



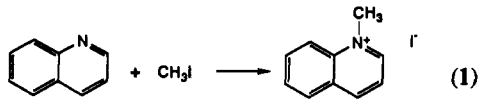
12–16 **17–21**

X	sulfonium salt	sulfide
NO_2	12	17
Cl	13	18
F	14	19
H	15	20
CH_3	16	21

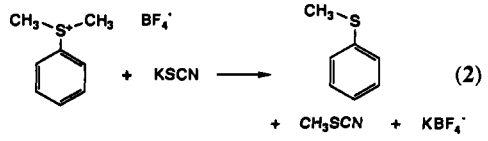
other things, that the cation–π interaction might be an especially effective force for catalysis. In the present work, we probe this interaction further by evaluating the effects of substrate variation on the catalytic efficiency of our cyclophane hosts. In addition, we establish that the same hosts can catalyze the dealkylation of onium compounds—the formal reverse of the Menschutkin reaction. Thus, the same simple host system can catalyze both reactions that create positive charge and those that destroy positive charge. The results are discussed in terms of several "effects", including the cation–π interaction, the hydrophobic effect, desolvation, and polarizability.

Results

I. Kinetic Scheme. We have studied two different types of reactions, both of which can be modeled by the same kinetic scheme. The first is the alkylation by methyl iodide of a quinoline-type compound (**3**, **5**, **7**, **9**, **11**) to form an *N*-methylquinolinium iodide or related structure (**4**, **6**, **8**, **10**) (eq 1). The

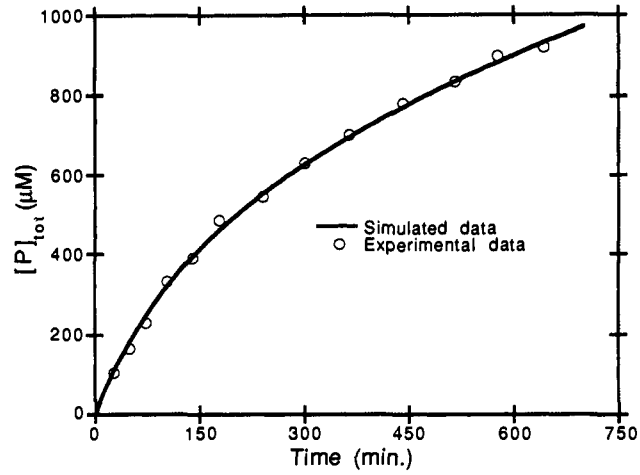


(1)



(2)

second is the dealkylation by thiocyanate of an aryldimethylsulfonium compound (**12–16**) to form methyl thiocyanate and the methyl aryl sulfides **17–21** (eq 2). Both systems can be analyzed with reference to the kinetic model of Scheme I. The uncatalyzed



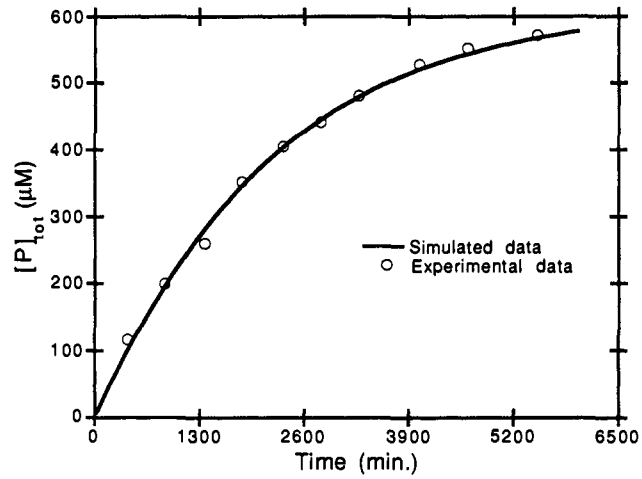


Figure 1. Simulation of experimental rate data for: top—reaction of **3** with methyl iodide in phosphate buffer; bottom—reaction of **12** with KSCN. Both reactions are catalyzed by host **1**. Relevant data are in Tables I and II.

reaction is a conventional S_N2 reaction with bimolecular rate constant k_{un}. Similarly, the catalyzed reaction is an S_N2 reaction between the "reagent" X (CH_3I or SCN^-) and the host–substrate complex H·S with bimolecular rate constant k_{cat}. Also relevant are the association constants of the substrate, K_s, and product, K_p.

A variety of observations support this overall scheme. First, Scheme I contains only one unknown, k_{cat}; all other parameters—k_{un}, K_s, and K_p—are independently determined with good accuracy. Thus, one can easily set up a numerical simulation, calculating the rise in product (P) as a function of time with k_{cat} as the only adjustable parameter. One determines k_{cat} directly by varying its value until the fit to the experimental data is optimized. The overall good quality of the fits (Figure 1) supports the general validity of the scheme. Note that product inhibition is predicted to be quite significant in the alkylation chemistry, since the product iminium ions are more tightly bound than the substrates. The simulation of Scheme I includes such effects, which are manifest as a decrease in apparent rate at long reaction times. In the best case, as many as five turnovers can be seen in the alkylation chemistry before product inhibition effectively shuts down the reaction. This effect is less pronounced in the dealkylation chemistry, where the substrate is bound more tightly than the product.

In addition, a number of control experiments support the kinetic scheme. First-order behavior in [X] (Scheme I) was established by varying [X] in the standard way. That the catalyzed reaction is first order in [H·S] is supported by two observations. The first is the goodness of the fits from the numerical simulations, which assume such behavior. The second is the response of the system

(7) See also: Schneider, H.-J.; Blatter, T.; Zimmermann, P. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1161–1162.
(8) Dougherty, D. A.; Stauffer, D. A. *Science* **1990**, *250*, 1558–1560.
(9) Sussman, J. L.; Harel, M.; Frolow, F.; Oefner, C.; Goldman, A.; Toker, L.; Silman, I. *Science* **1991**, *253*, 872. McLane, K. E.; Wu, X.; Conti-Tronconi, B. M. *Biochemistry* **1991**, *30*, 10730–10738. McLane, K. E.; Wu, X.; Conti-Tronconi, B. M. *Biochem. Biophys. Res. Commun.* **1991**, *176*, 11–17. Chaturvedi, V.; Donnelly-Roberts, D. L.; Lentz, T. L. *Biochemistry* **1992**, *31*, 1370–1375. Tomaselli, G. F.; McLaughlin, J. T.; Jurman, M. E.; Hawrot, E.; Yellen, G. *Biophys. J.* **1991**, *60*, 721–727. Cohen, J. B.; Sharp, S. D.; Liu, W. S. *J. Biol. Chem.* **1991**, *266*, 23354–23364. O'Leary, M. E.; White, M. M. *J. Biol. Chem.* **1992**, *267*, 8360–8365. Changeaux, J.-P.; Devillers-Thiéry, A.; Galzi, J.-L.; Bertrand, D. *Trends Pharm. Sci.* **1992**, *13*, 299–301.
(10) Stauffer, D. A.; Barrans, R. E., Jr.; Dougherty, D. A. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 915–918.
(11) Cachaza, J. M.; Herraez, M. A. *An. Quim.* **1971**, *67*, 11–16.
(12) Several previous examples of catalysis of S_N2 reactions have been reported, the accelerations being attributed to both "proximity" effects and electrostatic stabilization of developing charges. See, for example: Schmidtchen, F. P. *Top. Curr. Chem.* **1986**, *132*, 101–133. Schneider, H.-J.; Busch, R. *Angew. Chem.* **1984**, *96*, 910–911. Schneider, H.-J.; Busch, R. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 911–912. Kelly, T. R.; Zhao, C.; Bridger, G. J. *J. Am. Chem. Soc.* **1989**, *111*, 3744–3745.

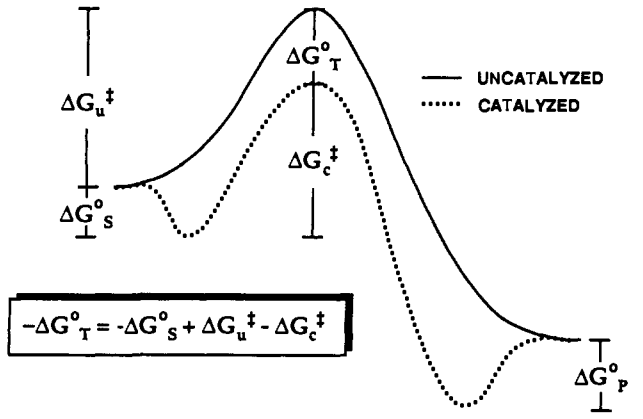
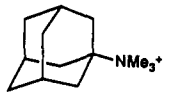


Figure 2. Schematic free energy diagram for catalyzed and uncatalyzed reactions.

to the addition of "competitive inhibitors". That is, addition of a nonreactive structure that binds to the host (adamantyltrimethylammonium (**22**), in the alkylation reactions or 5-nitroquinoline (**9**) in the dealkylation reactions) suppresses the apparent k_{cat} to an extent directly related to the amount of host occupied by the inhibitor. This establishes that binding to the host is



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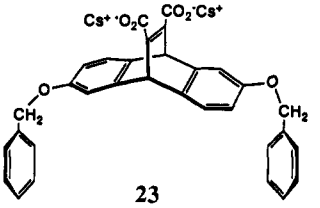

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essential for the catalysis. Also, no catalysis is seen if the host is replaced by the nonmacrocyclic structure **23**. In both the alkylation and dealkylation systems, we have sought, but failed to find, any evidence for either a bimolecular association between H and X or any type of termolecular H·S·X complex. All of the reactions are completely irreversible under the conditions used.

Scheme I presents the simplest mechanism that is consistent with our observations, and so we adopt it as our model for all the reactions we will discuss. It is fundamentally a Michaelis–Menten scheme[13] with some small but important distinctions. First, since we can independently determine K_s and K_p, all ambiguities concerning the interpretations and interrelations of the conventional Michaelis parameters (k_{cat} and K_m) are removed. Also, both k_{un} and k_{cat} are bimolecular rate constants for S_N2 reactions and so can be directly compared. Often in enzyme-mimetic systems, one must compare a bimolecular k_{un} with a unimolecular k_{cat}.

Figure 2 shows the free energy diagram implied by Scheme I, with rate constants and association constants converted to appropriate free energies. Simple arithmetic produces $\Delta G°_T$, the free energy of binding the transition state. Assuming that our mechanistic model is correct, there is no ambiguity as to the nature of this number—it is the free energy change on moving the reaction transition state out of aqueous buffer and into the host binding site.

II. Alkylation Chemistry. The alkylation of quinoline (**3**) with methyl iodide to form *N*-methylquinolinium iodide (**4**) is substantially accelerated by host **1**. The system is well modeled by Scheme I to give k_{cat}/k_{un} = 80 in pH = 9, 10 mM borate buffer.[14] Relevant data are summarized in Table I. Rate constants are given in the Experimental Section (Table II). With host **2**, k_{cat}/k_{un} is reduced to 20. Alkylation of isoquinoline (**5** → **6**) is also catalyzed by **1**, although to a lesser degree. Free energies of binding for the transition states, calculated according to the scheme of Figure 2, are also given in Table I.

Table I. Rate Enhancements and Binding Constants

substrate	product	host	k_{cat}/k_{un}	$-\Delta G_s^f$ (kcal/mol)	$-\Delta G_t^f$ (kcal/mol)	$-\Delta G_p^f$ (kcal/mol)
3[a]	**4**	**1**	80	5.7	8.3	8.4
5[a]	**6**	**1**	10	6.3	7.8	7.2
3[a]	**4**	**2**	20	5.9	7.7	6.6
7[b]	**8**	**1**	56	6.0	8.4	7.7
3[b]	**4**	**1**	29	5.2	7.2	7.4
9[c]	**10**	**1**	2	6.9	7.1	7.6
12[d]	**17**	**1**	3.3	5.7	6.5	4.4[e]
12[d]	**17**	**2**	9.4	4.5	5.9	4.4[e]
13[d]	**18**	**1**	2.6	6.2	6.8	4.4[e]
14[d]	**19**	**1**	2.3	5.5	6.0	3.9[e]
15[d]	**20**	**1**	2.0	5.3	5.7	3.9[e]
16[d]	**21**	**1**	1.9	5.9	6.3	4.4[e]

[a] 300 K, pD = 9 borate buffer. [b] 298 K, pD = 11.7 phosphate buffer. [c] 338 K, pD = 11.7 phosphate buffer. [d] 319 K, pH = 9 HPLC buffer. [e] Estimated. [f] Errors in ΔG values are ±0.2 kcal/mol.

Table II. Rate Constants[e]

substrate	host	k_{un} (M^{-1} s^{-1})	k_{cat} (M^{-1} s^{-1})
3[a]	**1**	2.40×10^{-5}	1.90×10^{-3}
5[a]	**1**	1.92×10^{-4}	2.10×10^{-3}
3[a]	**2**	2.40×10^{-5}	5.1×10^{-4}
7[b]	**1**	1.0×10^{-4}	5.6×10^{-3}
3[b]	**1**	9.4×10^{-5}	2.9×10^{-3}
9[c]	**1**	7.36×10^{-4}	1.53×10^{-3}
12[d]	**1**	1.20×10^{-4}	3.99×10^{-4}
12[d]	**2**	1.20×10^{-4}	1.13×10^{-3}
13[d]	**1**	8.18×10^{-6}	2.16×10^{-5}
14[d]	**1**	5.46×10^{-6}	1.25×10^{-5}
15[d]	**1**	4.32×10^{-6}	8.56×10^{-6}
16[d]	**1**	2.22×10^{-6}	4.18×10^{-6}

[a] 300 K, pD = 9 borate buffer. [b] 298 K, pD = 11.7 phosphate buffer. [c] 338 K, pD = 11.7 phosphate buffer. [d] 319 K, pD = 9 HPLC buffer. [e] Errors in k_{un} are <±5%, which is much smaller than errors in k_{cat}. We estimate errors in k_{cat} to be ±10%.

Since host **1** is a general receptor for positive charges of the sort present in **4** and **6**, it seems reasonable to assume that a significant component of the catalysis is due to the host binding the developing positive charge of the transition state. In order to probe this effect further, substrates with electron-donating or electron-withdrawing groups were desired. On the basis of synthetic availability and other considerations, quinolines **7** and **9** were chosen. The dimethylamino donor group of **7** increases the basicity of the quinoline such that **7** is completely protonated in our usual buffer. We thus changed to a higher pH buffer (pH = 11.7, 25 mM phosphate buffer). For comparison, we redetermined k_{cat}/k_{un} for **3** → **4** in the new buffer and found that the value decreased somewhat. However, this decrease is almost entirely due to an increase in k_{un} (Table II), presumably due to the higher ionic strength of the medium. The value of k_{cat} was essentially unchanged.

One would expect a donor substituent to increase k_{un}, while an acceptor substituent should produce a decrease. The effects we see on k_{un} are very small, consistent with previous studies[15] that suggest that substituent effects on such reactions are often small and do not always follow simple trends. Our results do clearly indicate that a donor substituent (**7**) also increases k_{cat}/k_{un}, while an acceptor (**9**) decreases it. Qualitatively, we observed that the acceptor-substituted quinoline **11** also showed a smaller k_{cat}/k_{un} than **3**, but the product of this reaction was insoluble in the medium and so quantitative studies were not possible.

III. Dealkylation Chemistry. An intriguing observation from the alkylation studies was that the binding constant for the

(13) See, for example: Walsh, C. *Enzyme Reaction Mechanisms*; Freeman: New York, 1979.

(14) More recent binding studies, including some using circular dichroism instead of NMR as the analytical tool, have led to an upward revision of the binding constants for **3** and **4** in borate buffer. These changes, which are included in Table I, lead to a small change in the previously reported[10] k_{cat} for this reaction. We thank J. Forman and P. Kearney for performing the new binding studies—full details will be reported elsewhere.

(15) Johnson, C. D.; Robert, I.; Taylor, P. G. *J. Chem. Soc., Perkin Trans. II* **1981**, 401. Fischer, A.; Galloway, W. J.; Vaughn, J. *J. Chem. Soc.* **1964**, 3596. Clarke, K.; Rothwell, K. *J. Chem. Soc.* **1960**, 1885.

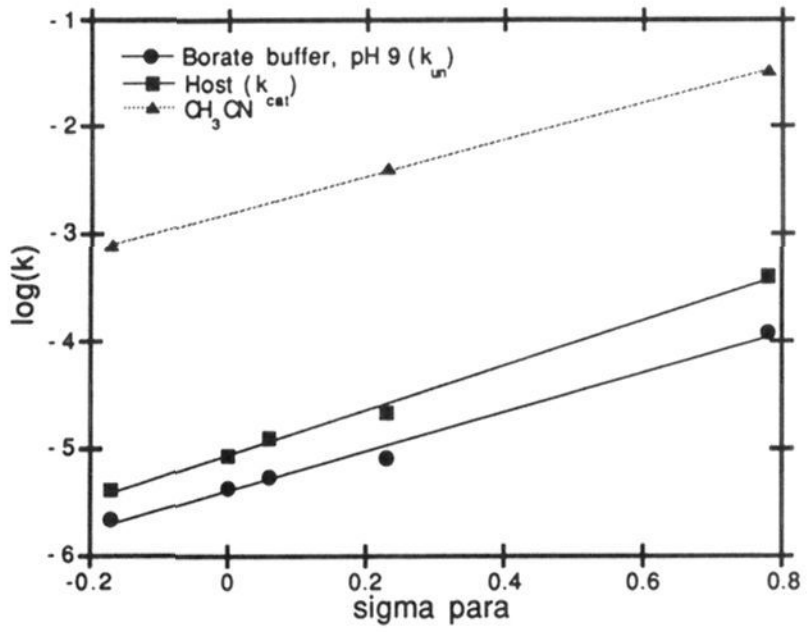


Figure 3. Hammett plots for sulfonium dealkylation reactions.

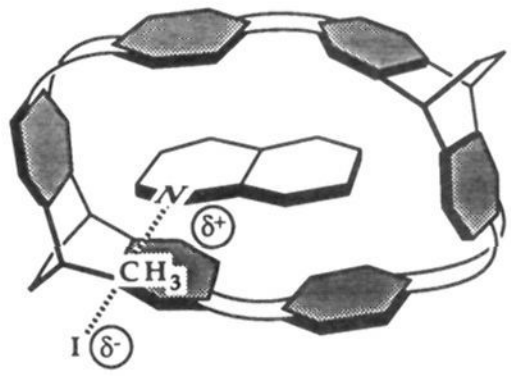
transition state (reflected in $\Delta G°_T$) could, in some cases, be calculated to be larger than that for substrate or product (Table I). This implied that the reverse reaction, the dealkylation of a quinolinium, could also be catalyzed by **1** and **2**. Unfortunately, these reactions are far too exothermic to be reversed under acceptable conditions. We thus sought an alternative system for testing the predicted catalysis of dealkylation chemistry.

We have now found that the dealkylation of aryldimethylsulfonium compounds is catalyzed by hosts such as **1** and **2** in pH = 9 borate buffer. The magnitudes of k_{cat}/k_{un} are generally smaller than those in the alkylation reactions, as anticipated on the basis of the $\Delta G°$ data. However, using HPLC to monitor the kinetics, we have obtained higher precision than was obtained with the NMR methods used for the quinoline chemistry. The data fit the model of Scheme I well, producing accurate values of k_{cat}/k_{un} across a series of sulfonium salts **12–16** (Table I). This produces a good fit to a Hammett plot for k_{cat} using σ_{para} values (Figure 3). For comparison, we have also produced Hammett plots for the uncatalyzed reaction in our buffer (i.e., k_{un}) and in acetonitrile as solvent (Figure 3). As expected, a donor substituent now slows the rates, while an acceptor produces an increase. This is in agreement with previous studies of substituent effects on aryldimethylsulfonium salt demethylations in water and acetonitrile, which also show a correlation with σ_{para}.[16] In fact, the previous acetonitrile study gave the same ρ (1.7) for the reaction of similar sulfoniums with hydroxide.[16] In parallel with the alkylation chemistry, the response of k_{cat}/k_{un} to substituents is in the same direction as that of the absolute rate constants.

Discussion

Catalysis of Alkylation. Host **1** can substantially accelerate the alkylation of quinolines by methyl iodide. Note that in our model, *none of the rate enhancement is due to proximity effects*, i.e., the entropic advantage of bringing together two reaction partners. In most biomimetic systems, an entropy effect dominates the catalysis, making analysis of electronic effects on catalysis problematical. In the present system, however, both the catalyzed and uncatalyzed alkylations are bimolecular S_N2 reactions. The only difference is that in one case the nucleophile is the quinoline and in the other it is the host–quinoline complex. Based on NMR data,[6] computer modeling, and crystallography of the host,[17] we assume a reactive geometry as shown schematically in **24**. This makes the N of the quinoline quite accessible,[18] and so the "supramolecule" is the nucleophile.

Our kinetic scheme assumes that the complex probed by equilibrium binding studies is catalytically productive. If that is not the case (i.e., there is nonproductive binding), then the



24

binding constant for the productive complex must be smaller than the measured value. Inserting a less negative ΔG_s into our kinetic scheme must produce a larger k_{cat}, and so our reported k_{cat}/k_{un} values can be considered lower limits.

It is important to appreciate that the catalysis we see in the alkylation chemistry is *not* just a hydrophobic effect. It is well established that Menschutkin reactions are *faster* in aqueous media than in conventional organic solvents.[11] We have verified this in the present system by determining that the uncatalyzed reaction of **3** with methyl iodide is faster in the pH = 9 buffer than in either benzene or chloroform. Also, increasing the ionic strength of an aqueous medium increases the rate.

Thus, the alkylation catalysis we see is best explained by a preferential binding of the transition state (relative to substrate) by the host due to an effect that is neither a proximity nor a hydrophobic effect. Of course, the cation–π interaction is a prime candidate for such an effect. Hosts such as **1** and **2** preferentially bind positive charges, and these reactions develop positive charge in their transition states. The cation–π effect is typically larger in host **1** than in host **2**, and indeed, host **1** is the better catalyst. Thus, it seems safe to conclude that a major factor in these systems is the recognition by the hosts of the developing positive charge in the transition state, which produces a preferential binding of the transition state and thus catalysis.

To the extent that catalysis is a consequence of binding positive charge in the transition state, an increase in this charge should produce a greater catalytic effect. This was the motivation for the study of the series **3**, **7**, **9**. Because the substituents are not in the same position and because the precision of these k_{cat}/k_{un} values is not high, a quantitative Hammett plot is not feasible. However, the qualitative conclusion is clear. The electron-donating substituent **7** increases k_{cat}/k_{un}, while the electron-withdrawing substituent **9** decreases it. The donor-substituted quinoline **7** should be a better nucleophile than the parent **3**, so a Hammond-type argument would suggest an earlier transition state and thus *less* positive charge developed at N. This provides one indication that some factor in addition to simple charge recognition is operative in the catalysis.

Catalysis of Dealkylation. An initially surprising observation from the alkylation studies was that $-\Delta G°_T$ could be *larger* than $-\Delta G°_P$. That is, the transition state, with only a partial positive charge, can be more tightly bound than the product, with a full positive charge. This is a second indication that simple charge recognition is not solely responsible for catalysis. Of course, an immediate implication of this analysis is that the reverse reaction, the dealkylation of a quinolinium, should also be catalyzed by **1** and **2**. Since $-\Delta G°_T$ is a somewhat derived number and since not all systems show this effect, we felt it was important to independently verify this important conclusion. Since quinoliniums cannot be demethylated under any practical conditions, we sought an alternative model system. The demethylation of sulfonium salts **12–16** has served that purpose.[19]

Hosts **1** and **2** do catalyze the dealkylation of **12–16**. The effects are generally not large, but this was anticipated. As with the alkylation chemistry, there are no favorable entropic terms associated with this catalysis. Also, considering the **5**/**6** system in

(16) Coward, J. K.; Sweet, W. D. *J. Org. Chem.* **1971**, *36*, 2337–2346.
(17) Forman, J. F.; Marsh, R. E.; Schaefer, W. P.; Dougherty, D. A. In press.
(18) For isoquinoline in a similar geometry, the N would be much less accessible, perhaps contributing to the smaller k_{cat}/k_{un}.
(19) For general treatments of sulfonium salts and their reactivity, see: Stirling, C. J. M. In *Organic Chemistry of Sulfur*; Oae, S., Ed.; Plenum Press: New York, 1977; Chapter 9.

10 mM buffer, one expects the catalysis of dealkylation to be worth only 7.8–7.2 = 0.6 kcal/mol. The rate enhancements from host **1** for **12–16** correspond to $\Delta\Delta G^{\circ}_{T\text{-}S}$ in the 0.5–0.8 kcal/mol range (recall T = 45 °C), perhaps coincidentally close to expectation. This does suggest, though, that the sulfonium chemistry is a good model for quinolinium dealkylation. Further support for this conclusion comes from the effect of host **2**: it gives the largest dealkylation k_{cat}/k_{un} we have seen (9.4 for **12**, corresponding to $\Delta\Delta G^{\circ}_{T\text{-}S}$ = 1.4 kcal/mol). Remarkably, this result was presaged by our alkylation studies, in that host **2** is a poorer catalyst for **3** → **4**, but the thermodynamics suggested that the reverse reaction should be more effectively catalyzed by **2** (7.7–6.6 = 1.1 kcal/mol), and this is the case.

A complicating factor in the interpretation of the dealkylation chemistry is that this reaction might be expected to go faster in a nonpolar environment than in water, since charge is being destroyed. Such effects are seen, but a careful analysis[20] reveals that the dominant factor is the enhanced reactivity due to desolvation of the nucleophile (in this case SCN^-) on going from a hydrogen-bonding solvent like water to an aprotic solvent. However, in our system this effect is absent, since the nucleophile is not complexed by the host. A comparison of reaction rates within a series of polar aprotic solvents can minimize the effect of nucleophile desolvation. This has been done for the reaction of Br^- with Me_3S^+, and the rate as a function of solvent follows the sequence CH_3NO_2 > dimethylacetamide (DMA) > DMF, while the solvent polarity (E_T) sequence is CH_3NO_2 > DMF > DMA. The reaction is actually fastest in the most polar solvent, but a simple trend is not observed. Parker has carried out a more detailed analysis of this reaction[20] which allows one to quantitatively factor out the solvation of the nucleophile across a range of solvents, including the hydrogen-bonding solvent ethanol. This analysis indicates that, after factoring out nucleophile solvation, the Br^- plus Me_3S^+ reaction does correlate with E_T and goes faster in *more polar* solvents. We conclude, then, that the catalysis we observe is not simply a consequence of moving the reaction out of the more polar aqueous environment and into the less polar host.

The Hammett analysis of the dealkylation chemistry is more quantitatively reliable than that for the alkylation chemistry for several reasons: a more consistent series of substrates was available, thiocyanate does not escape the reaction vessel over time like the volatile methyl iodide does, the HPLC method was more quantitatively reliable than the NMR, and product inhibition was not an issue, since the neutral sulfides bind only poorly to the hosts. Again, the Hammett plots suggest that catalysis is more effective for an early transition state, but in this case, early implies *more* positive charge in the transition state. This is thus different from what is seen in the alkylation chemistry. The Hammett plots also provide a second way to consider solvation effects. Considering the plots of k_{cat}(host), k_{un}(buffer), and k_{un}(CH_3CN), there are differences in the slopes (Figure 3), and the slope for the catalyzed reaction differs from that for the uncatalyzed (in buffer) in the *opposite* direction than does that for acetonitrile. The differences are small, but the results again suggest that the effect of the host is something other than simply moving the transition state into a more hydrophobic environment.

Polarizability. The results of the various studies described above suggest that some additional factor is involved in the preferential binding of transition states exhibited by host **1**. It is difficult to imagine that the anionic group (I^- or SCN^-) plays a large role in transition-state binding, given the expected reactive geometry (**24**) and the well-documented preference of these hosts for cationic guests.[6] We have attempted to prove this effect experimentally by varying the leaving group in the alkylation chemistry, but we have been unable to find another alkylating agent that is reactive enough yet is stable in our buffers.

Certainly charge recognition is important. But it seems insufficient to explain the preference for partially charged transition states over fully charged structures, the preference for earlier alkylation transition states over later ones, and the Hammett plots in the sulfonium system. Charge recognition is a simple electrostatic effect—the negative face of the aromatic rings of **1** interacting with the positive charge of the transition state. This is basically a Coulombic term, and such effects do not "saturate". That is, in considering the interaction of two (opposite) charges, it is *always* true that the more charge the better—the Coulombic interaction never "turns over". The same is true for charge–dipole and charge–quadrupole interactions.[21]

This suggests an interaction that favors the intermediate-type structure of a transition state, and we propose that polarizability is such a factor. The long, weak bonds of a transition state are more polarizable than the full bonds of substrate and product. The hosts **1** and **2** are also quite polarizable, and so a large London dispersion energy, which depends strongly on the polarizability of the interacting partners, is expected.

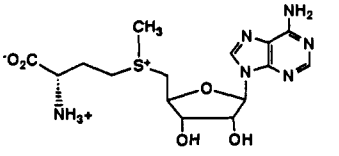
Water, in contrast to our host cavity, is polar but not polarizable. Water solvates charge by orienting its molecular dipole properly. This is a relatively slow process, and the large dielectric constant associated with water is the low-frequency dielectric constant, which corresponds to slow timescales. In contrast, water's high-frequency dielectric constant, which correlates directly with polarizability, is quite low. For example, the high-frequency dielectric constant—which is just the square of the refractive index—is larger for hexane than it is for water. Since the "lifetime" of a transition state is short, presumably the high-frequency dielectric is more important, and the face of a benzene ring is far superior to water.

Our observations concerning substituent effects suggest a complex interplay of forces. In both the alkylation and dealkylation systems, the host enhances the ability of substituents to accelerate the reaction. That is, k_{cat}/k_{un} has the same sign for the slope of a Hammett plot as does k_{un}. We believe that both charge recognition and polarizability are important in rationalizing substituent effects. We assume that substituents that delocalize charge also increase the polarizability of the transition state. In the alkylation chemistry, apparently the polarizability term dominates, since the preferred substituent NMe_2 leads to less charge but more charge dispersion in the transition state. In the dealkylation chemistry, the favorable substituent NO_2 produces both more charge and more charge dispersion in the transition state, and so both effects operate in the same direction. It is still necessary, though, to invoke polarizability, because if only charge recognition were involved the substrate would be bound more tightly than the transition state.

Biological Relevance

Our hosts were not designed to explicitly mimic any known enzyme active site.[22] Rather, they are generic receptors, designed to allow detailed study of the forces involved in molecular recognition. There is, however, a broad class of enzymes that mediate reactions similar to those studied here, and it is interesting to speculate on any possible connections between the two.

Enzymatic methylation is an important and pervasive regulatory process in living systems, and the majority of methylations involve the sulfonium compound S-adenosylmethionine (SAM) as the methylating agent.[24] The transition states of many SAM-me-



SAM

(20) Parker, A. J. *Chem. Rev.* **1969**, *69*, 1–32.

(21) Israelachvili, J. N. *Intermolecular and Surface Forces*; Academic Press: New York, 1985.

(22) The catalysis is, nevertheless, "biomimetic". Recall that, as originally defined by Breslow,[23] biomimetic chemistry involves imitating the "style" of biological chemistry rather than precisely duplicating a known enzymatic reaction.

(23) Breslow, R. *Chem. Soc. Rev.* **1972**, *1*, 533. Breslow, R. *Isr. J. Chem.* **1979**, *18*, 187–191.

diated reactions are quite similar to those studied here, and so it seems plausible to invoke cation–π and polarization effects at the active sites. Little structural information is available for enzymes that involve SAM.[25] The methylation enzymes are for the most part membrane bound, suggesting that the chemistry occurs in a relatively hydrophobic environment. We speculate that the active sites of such enzymes will be rich in aromatic rings, much like acetylcholine binding sites are.[8,9]

Conclusions

Our studies have shown that relative to an aqueous medium host **1** presents a superior environment for both alkylation and dealkylation reactions involving onium compounds. Catalytic rate enhancements are not large but are significant considering the lack of any entropy contribution. One could argue that such systems allow a more nearly direct measurement of the enthalpic stabilization of the transition state provided by the catalyst.

A combination of several factors is responsible for the catalysis we see. Of course, cyclophane hosts provide a hydrophobic environment that binds organic molecules in a generic fashion. The issue with catalysis, though, is not binding but rather differential binding of the transition state relative to the substrate, as Pauling so clearly recognized over 45 years ago.[26] It seems unlikely that hydrophobic effects are solely responsible for such discrimination in these systems.

Certainly, the cation–π interaction is important in the catalysis we see. The biggest rate enhancements are seen in the Menschutkin reactions, which involve the development of a positive charge in the transition state. To first order, the best way to think of this interaction is as an electrostatic effect. The face of a benzene ring is, in effect, negatively charged and so interacts favorably with a positive charge. Formally, this is a charge–quadrupole interaction, but it is perhaps simpler to think of it as a simple Coulombic interaction between the "anionic" benzene face and the cation. We believe nature can use an aromatic ring as a novel "hydrophobic negative charge" in several contexts but especially in membrane-bound receptors and catalysts.

The electrostatic view of the cation–π interaction requires that the effect cannot "saturate"—it is always true that an increase in charge will lead to an increase in the interaction. The present studies, though, clearly indicate that maximizing positive charge development is not always favorable for binding a transition state. This leads us to propose that London dispersion terms, which scale directly with the polarizabilities of the interacting partners, are also important in the catalysis we see. Transition states should be especially polarizable because of the forming and breaking of bonds and the dispersal of charge generally involved. Water is polar but not polarizable, and so an alternative medium that combines electrostatic charge stabilization plus polarizability can be superior to water for many types of reactions.

The role of the host, then, is to surround the transition state with electron-rich aromatic rings *in the proper orientation.* Pure benzene as a solvent is a less favorable environment than the host because at the instant the transition state forms, it is unlikely that the first solvation shell will be perfectly aligned to maximize the favorable interactions. This is a very simple type of catalysis, and we believe that similar environments could be present at the active sites of certain enzymes.

Experimental Section

Uncorrected melting points were recorded on a Thomas-Hoover melting point apparatus. ^1H and ^{13}C NMR spectra were recorded on JEOL JNM GX-400 and Bruker AM500 spectrometers. Routine spectra were referenced to the residual proton and carbon signals of the solvents and are reported (ppm) downfield of 0.0 as δ values, except where noted. Infrared spectra were recorded on a Perkin-Elmer Model 1600 FT-IR spectrophotometer. Elemental analysis was performed at Galbraith Laboratories, Inc., Knoxville, TN. E. Merck silica gel 60, 0.04–0.063 mm, was used as packing material in column chromatography. Chromatographic eluents are reported as volume-to-volume ratios (v/v). HPLC was performed on a Waters dual 510 pump liquid chromatograph system equipped with a Waters 490E variable wavelength UV detector and a Phase Separations Spherisorb ODS1 column (25 cm × 4.6 mm). Solvents used for HPLC were spectrophotometric grade acetonitrile (Burdick and Jackson) and doubly distilled water passed through a Milli-Q filtration system.

Tetrahydrofuran (THF) was dried over sodium benzophenone ketyl under a nitrogen atmosphere. Quinoline was distilled before use. Millipore-filtered water was used as eluent for ion-exchange columns. Quinoline, isoquinoline, 5-nitroquinoline, 4-chloroquinoline, 1-methylquinolinium iodide, trifluoroacetic acid (TFA, spectrophotometric grade), potassium thiocyanate (KSCN), thioanisole, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), 3,3-dimethylglutarate (DMG), and potassium hydrogen phthalate (KHP) were commercially available. Hosts **1**, **2**, and *N*-methylisoquinolinium iodide were synthesized as described previously.[6] The ion-exchange resin for changing iodides to chlorides was Dowex-1-chloride, 8% cross-linking, 200–400 dry mesh.

4-(Dimethylamino)quinoline (7).[27] A total of 953 mg (5.8 mmol) of 4-chloroquinoline, 3.7 g of pulverized 87% potassium hydroxide pellets, and 20 mL of DMF were added to a 50-mL round-bottomed flask, and the mixture was refluxed for 20 h. The heterogeneous solution was then allowed to cool to room temperature. A total of 100 mL of water was added to the reaction mixture, and the mixture was extracted with three 50-mL portions of dichloromethane. The dichloromethane portions were combined, and the organic phase was then washed three times with water and dried with magnesium sulfate. Chromatography (1000 mL of 98:2 CH_2Cl_2:MeOH, 900 mL of 95:5 CH_2Cl_2:MeOH) gave 530 mg (53%) of a yellow oil: ^1H NMR (500 MHz, $CDCl_3$ referenced to tetramethylsilane) δ 8.66 (d, J = 5.1 Hz, 1 H), 8.06 (d, J = 8.5 Hz, 1 H), 8.03 (d, J = 8.5 Hz, 1 H), 7.64 (t, J = 8.5 Hz, 1 H), 7.46 (t, J = 8.5 Hz, 1 H), 6.76 (d, J = 5.1 Hz, 1 H), 3.05 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 157.62, 150.16, 149.37, 129.63, 129.43, 128.93, 124.54, 124.36, 107.11, 43.85; MS (EI) m/z (relative intensity) 172 (100, M^+), 157 (4, M^+-CH_3), 129 (3, $M^+-C_2H_5N$); HRMS 172.1002, calcd for $C_{11}H_{12}N_2$ 172.2317.

Quinolinium Chlorides. The quinolinium chlorides were obtained by stirring the quinolines in excess methyl iodide at room temperature for 16–24 h. The reaction vessel was wrapped in aluminum foil. The solvent was removed in vacuo, and the quinolinium salt was washed several times with ether. The quinolinium iodides were then eluted through an ion-exchange column using doubly distilled water. The fractions containing UV-visible material were collected and lyophilized overnight. The quinolinium chlorides were dried at 82 °C under vacuum (1 Torr) for 24 h.

1-Methyl-4-(dimethylamino)quinolinium chloride (8): mp 121–123 °C: ^1H NMR (500 MHz, pD = 11.7 phosphate buffer, referenced to DMG) δ 8.39 (d, J = 8.5 Hz, 1 H), 8.25 (d, J = 7.5 Hz, 1 H), 7.98 (m, 2 H), 7.69 (t, J = 7.5 Hz, 1 H), 6.85 (d, J = 7.5 Hz, 1 H), 4.11 (s, 3 H), 3.48 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 160.21, 147.51, 140.04, 133.86, 127.58, 125.67, 117.68, 104.007, 44.64, 42.53; MS (EI) m/z (relative intensity) 187 (37, M^+), 172 (86, M^+-CH_3), 171 (100, M^+-CH_4).

1-Methyl-5-nitroquinolinium chloride (10): mp 212–213 °C; ^1H NMR (500 MHz, CD_3CN) δ 9.58 (d, J = 8.8 Hz, 1 H), 9.37 (d, J = 5.3 Hz, 1 H), 8.72 (d, J = 9.0 Hz, 1 H), 8.68 (d, J = 8.0 Hz, 1 H), 8.35 (dd, J = 8.8, 8.0 Hz, 1 H), 8.22 (dd, J = 9.0, 5.3 Hz, 1 H), 4.66 (s, 3 H); ^{13}C NMR (D_2O, external TSP-d_4 at 0.0 ppm) δ 153.80, 145.96, 137.00, 130.63, 127.70, 127.02, 125.58, 49.63; MS (EI) m/z (relative intensity) 189 (11, M^+), 174, (31, M^+-CH_3), 143 (40, M^+-NO_2), 128 (100, $M^+-CH_3NO_2$).

1-Methyl-4-(1-oxopropyl)quinolinium chloride: mp 181–182 °C; ^1H NMR (500 MHz, CD_3CN) δ 9.52 (d, J = 6.0 Hz, 1 H), 8.43 (d, J = 9.0 Hz, 1 H), 8.32 (d, J = 8.5 Hz, 1 H), 8.27 (dd, 1 H), 8.16 (d, J = 6.0 Hz, 1 H), 8.03 (dd, 1 H), 4.64 (s, 3 H), 3.14 (q, J = 6.9 Hz, 2 H), 1.24 (t, J = 6.9 Hz, 3 H); MS (EI) m/z (relative intensity) 200 (100, M^+), 186 (21, M^+-CH_2), 143 (50, $M^+-C_3H_5O$).

Alkyl Aryl Sulfides. The sulfides were all prepared from the appropriate thiophenol, alkyl halide, and DBU in benzene or petroleum ether as described in the chemical literature.[28] The reaction mixture was filtered and the filtrate was purified by column chromatography after the solvent was removed in vacuo.

Ethyl *p*-nitrophenyl sulfide: mp 41–44 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, J = 8.8 Hz, 2 H), 7.30 (d, J = 8.8 Hz, 2 H), 3.04 (q,

(24) Cantoni, G. L. *Ann. Rev. Biochem.* **1975**, *49*, 435–451. Maw, G. A. In *The Chemistry of the Sulphonium Group*; Stirling, C. J. M., Patai, S., Eds.; John Wiley & Sons Ltd: New York, 1981; Chapter 17. Lederer, E. Q. *Rev. Chem. Soc.* **1969**, *23*, 453–481. See also ref 13, pp 851–863.

(25) There is, however, a structure of the *Met* repressor with SAM bound as a corepressor. Rafferty, J. B.; Somers, W. S.; Saint-Girons, I.; Phillips, S. E. V. *Nature* **1989**, *341*, 705–710.

(26) Pauling, L. *Chem. Eng. News* **1946**, *24*, 1375–1377. Pauling, L. *Nature* **1948**, *161*, 707–709. Kraut, J. *Science* **1988**, *242*, 533–540.

(27) Balkav, F.; Elmes, B.; Loder, J. *Aust. J. Chem.* **1969**, *22*, 2489–2492.

(28) Ono, N.; Miyake, H.; Saito, T.; Kaji, A. *Synthesis* **1980**, 952.

J = 7.3 Hz, 2 H), 1.38 (t, J = 7.3 Hz, 3 H).

Methyl *p*-nitrophenyl sulfide (17): mp 68–71 °C: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, J = 9.0 Hz, 2 H). 7.27 (d, J = 9.0 Hz, 2 H), 2.53 (s. 3 H).

Methyl *p*-chlorophenyl sulfide (18): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (d, J = 8.6 Hz, 2 H), 7.14 (d, J = 8.6 Hz 2 H), 2.42 (s, 3 H).

Methyl *p*-fluorophenyl sulfide (19): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (m, 2 H), 6.97 (m, 2 H), 2.45 (s, 3 H).

Methyl *p*-tolyl sulfide (21): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (d, J = 8.1 Hz, 2 H). 7.09 (d, J = 8.3 Hz, 2 H), 2.45 (s, 3 H), 2.30 (s, 3 H).

Dialkylarylsulfonium Tetrafluoroborate Salts. The sulfonium salts were all prepared from stirred, refluxing mixtures of alkyl aryl sulfide and trimethyloxonium tetrafluoroborate in methylene chloride[29] (which was distilled from CaH_2). The reaction continued overnight, after which the solvent was removed in vacuo and the residue was partitioned between acetonitrile and petroleum ether and washed two more times with petroleum ether. The acetonitrile was removed in vacuo, and the solid was triturated twice from acetonitrile with diethyl ether.

Ethyl(*p*-nitrophenyl)sulfonium tetrafluoroborate: ^{1}H NMR (400 MHz, acetone-d_6) δ 8.59 (d, J = 9.1 Hz, 2 H), 8.50 (d, J = 9.1 Hz, 2 H), 4.05 (m, 1 H), 3.98 (m, 1 H), 2.81 (s, 3 H), 1.47 (t, J = 7.4 Hz, 3 H).

Dimethyl(*p*-nitrophenyl)sulfonium tetrafluoroborate (12): mp 116–120 °C; ^{1}H NMR (CD_3CN) δ 8.45 (d, J = 8.8 Hz, 2 H), 8.17 (d, J = 9.1 Hz, 2 H), 3.23 (s, 6 H); FAB-MS m/e 184 (M^+), 445 ($2M^+ + BF_4^-$); HRMS 184.0440, calcd for $C_8H_{10}NO_2S$ 184.0432.

Dimethyl(*p*-chlorophenyl)sulfonium tetrafluoroborate (13): mp = 112–118 °C; ^{1}H NMR (CD_3CN) δ 7.89 (d, J = 8.7 Hz, 2 H), 7.71 (d, J = 8.8 Hz, 2 H), 3.13 (s, 6 H); FAB-MS m/e 173 (M^+), 433 ($2M^+ + BF_4^-$); HRMS 173.0192, calcd for $C_8H_{10}ClS$ 173.0192.

Dimethyl(*p*-fluorophenyl)sulfonium tetrafluoroborate (14): mp 165–170 °C; ^{1}H NMR (CD_3CN) δ 7.97 (m, 2 H), 7.46 (m, 2 H), 3.11 (s, 6 H); FAB-MS m/e 157 (M^+), 401 ($2M^+ + BF_4^-$); HRMS 157.0472, calcd for $C_8H_{10}FS$ 157.044 87.

Dimethylphenylsulfonium tetrafluoroborate (15): mp 128–132 °C; ^{1}H NMR (acetone-d_6) δ 8.16 (m, 2 H), 7.81 (m, 3 H), 2.85 (s, 6 H); FAB-MS m/e 139 (M^+), 365 ($2M^+ + BF_4^-$); HRMS 139.055 72, calcd for $C_8H_{11}S$ 139.0581.

Dimethyl-*p*-tolylsulfonium tetrafluoroborate (16): mp 100–104 °C; ^{1}H NMR (CD_3CN) δ 7.78 (d, J = 8.5 Hz, 2 H), 7.51 (d, J = 8.3 Hz, 2 H), 3.09 (s, 6 H), 2.44 (s, 3 H); FAB-MS m/e 153 (M^+), 393 ($2M^+ + BF_4^-$); HRMS 153.0475, calcd for $C_9H_{13}S$ 153.0738.

Buffers. Binding studies and kinetics studies were performed in three different buffers: 10 mM pD = 9 borate; 25 mM pD = 11.7 phosphate; and HPLC 10 mM pH = 9 borate buffers. The pD = 9 borate buffer was prepared by dissolving 32 mg of high-purity boric oxide (B_2O_3) in 100 g of D_2O (Aldrich, 99.8 atom %D), adding enough CsOD in D_2O (e.g., 400 μL of 1 M CsOD) to attain pD = 9, and mixing thoroughly.[6c] The phosphate buffer was prepared by adding 2.5 mL of 1.0 M Na_2DPO_4 in D_2O, 1.11 mL of 1.0 M CsOD in D_2O, and 6.1 mL of D_2O to 100 g of D_2O and mixing thoroughly.[30] The pD of the phosphate buffer was determined using an Orion Model SA 720 pH meter with Lazar semimicro combination pH electrode (model H3800-1). The HPLC pH = 9 borate buffer was made by using doubly distilled water passed through a Milli-Q filtration system instead of using the D_2O used to make the pD = 9 borate buffer.

Binding and Kinetic Studies. Stock solutions for host (~1.6 mM), guest (~2–7 mM), KSCN (0.426 M), DMG, and KHP (9.25 mM) for the ^{1}H NMR binding and the kinetics experiments were prepared from the three buffers described above. Enantiomerically pure hosts were used in all binding and kinetic studies. All volumetric measurements of aqueous solutions were made using adjustable volumetric pipets. Concentrations for binding studies were determined by NMR integrations versus the internal standard (DMG) of known concentration. All pulse delays for the integration experiments were at least 5 times the measured T_1 for the species involved. Binding constants were determined by performing a ^{1}H NMR titration of guest added to host using the chemical shifts referenced to internal DMG (1.09 ppm) at 8–10 different guest:host concentration ratios in an iterative least-squares fitting procedure.[6c] Binding studies of product sulfides (**17–21**) were not possible due to low solubility in the buffer. All binding studies of compounds were performed at 400 MHz except for those in pD = 11.7 phosphate buffer, which were performed at 500 MHz. Binding constants are reported in Table I. The second-order rate constants for reactions were determined under pseudo-first-order conditions, as described in detail below for each study. The rate constant k_{un} combined with binding constants of substrate and product and rate data with host present allow determination of k_{cat} using a simulation program based on Scheme I. Table II contains rate constants.

Kinetics of Quinoline Alkylation, pD = 9. NMR tubes, which served as reaction vessels in the kinetics experiments, were made by John Pirolo in the Caltech Chemistry Glass Shop: half-dram screw-cap vials were fused (and balanced) to the tops of Norell 508-UP NMR tubes. The vial portion could then be capped and sealed using the plastic screw-caps in tandem with Teflon-lined silicone septa such that the volatile alkylating agent (iodomethane) could be maintained.

The standard NMR kinetics experiment was prepared by locking and manually shimming the sample, adjusting the probe temperature, and setting the pulse delay to 21 s and the number of scans to 64. The total accumulation time was 26–27 min per spectrum (data point).

For the kinetics experiments in 10 mM pD = 9 buffer solutions containing substrate, DMG and hosts **1** and **2** (for catalyzed samples) were introduced into the reaction vessels and buffer was added to give a total volume of 500 μL: the vessels were capped and sealed and then cooled in an ice-water bath. Iodomethane (2.0–3.5 μL, ca. 30–55 mM) was then injected through the septum with a 10-μL syringe. The cold solution was mixed by vigorous shaking. The reaction mixture was then recooled as the punctured septum was replaced with a new one. The cold reaction mixture was briefly sonicated to remove air bubbles and to complete mixing before the sample was placed into the NMR probe.

Relative concentrations of substrate and product in the Menschutkin reactions were monitored by 400-MHz ^{1}H NMR (JEOL JNM GX-400). Concentrations were determined by careful integration of appropriate peaks. The following protons were integrated to obtain substrate and product concentration: for uncatalyzed alkylation, H_2 (**3**), H_2 and H_4 (**10**), H_1 (**6**), H_1 (**11**); and for catalyzed alkylation, H_2 (**3**), N-CH_3 (**10**), H_1 (**6**), N-CH_3 (**11**). Initial concentrations of substrate and host were assumed from their respective stock solution concentrations as determined by ^{1}H NMR integration versus DMG. DMG also was employed as an integration standard to determine the average concentration of iodomethane during the course of the experiment. The reaction temperature was maintained and calibrated versus a methanol standard.

Kinetics of Quinoline Alkylation, pD = 11.7. The NMR tubes used for the kinetic investigations of the Menschutkin reaction were equipped with J. Young valves to minimize the loss of iodomethane over the course of a kinetics experiment.

The concentration of iodomethane present at a given time t was monitored by measuring the integrated areas of the methyl peak of DMG at 1.09 ppm and the peak at 2.17 ppm, which consisted of the combined areas of the methylene peak of DMG and the iodomethane singlet. There was typically a loss of 8–12% of the original iodomethane concentration over the course of 12 h at 298 K. The iodomethane concentration used to calculate the catalyzed and uncatalyzed rate constants was the average concentration of iodomethane in solution at the probe temperature during the time the reaction was monitored.

For the host-catalyzed kinetic experiments, the NMR tube was filled with 20 μL of 29.3 mM DMG, 90 μL of 1.8 mM host **1**, the necessary volume of quinoline stock solution to give a final quinoline concentration of 2.26 mM, and the appropriate volume of pD = 11.7 phosphate buffer to give a total volume of 500 μL. This resulted in a guest:host ratio of 7:1. For the uncatalyzed reaction, the 90 μL of host solution was replaced by pD = 11.7 phosphate buffer.

A kinetics experiment was initiated by placing the NMR tube in the probe of the Bruker AMX500 spectrometer, which had been preheated to the appropriate temperature. The lock signal was shimmed, and then the NMR tube was removed and placed in an ice bath. After the tube was cooled for 5 min, the Teflon piston was opened, 3.0 μL of iodomethane was added, and then the Teflon piston was closed. The tube was inverted 5 times, the top was wrapped with Parafilm, and the tube was replaced in the probe. Time zero was defined as the time the sample was dropped into the probe. A delay time of 3.5 s and 128 scans were used in order to assure very reproducible integrations. For the evaluation of the Menschutkin reaction, the change in integrated intensity of the proton signals of the substrate and product was followed. For the host-catalyzed methylation of **7**, the integral areas of the dimethylamino groups of **7** and **8** were measured as the substrate and product peaks, respectively. During the course of the methylation reaction at 298 K, two peaks at 3.45 and 3.47 ppm appear and grow in intensity. These peaks are presumably due to methylated phosphate species, since the addition of iodomethane to an NMR tube containing only the pD = 11.7 phosphate buffer and DMG also results in the appearance of these peaks. For the uncatalyzed methylation of **7**, the integral areas of the dimethylamino peak of **7** at 3.06 ppm and the methyl peak of **8** at 4.11 ppm were used as the substrate and product peaks. For the catalyzed and uncatalyzed methylation of

(29) Corey. E. J.; Jautelat. M.; Oppolzer. W. *Tetrahedron Lett.* **1967**, 2325.

(30) *CRC Handbook of Chemistry and Physics*. 1980–81 ed.; Weast. R., Ed.; CRC Press: Boca Raton. FL. 1980.

3, the integral areas of the 2-hydrogen of 3 and the methyl group of 4 were used as the substrate and product peaks, respectively.

Kinetics of Sulfonium Salt Dealkylation. Stock solutions of host (1.49 mM) and KSCN (0.426 M) were made in 10 mM deuterated cesium borate buffer at pD = 9. Stock solutions of the internal integration standard KHP (9.25 mM), 5-nitroquinoline (1.38 mM), and the sulfonium salts (3.8–6.8 mM) for HPLC studies were made by weighing each solid and dissolving it in 10.0 mL of HPLC borate buffer in a 10-mL volumetric flask. The reaction rates were monitored by integration of substrate and internal standard peak areas from an HPLC trace using a Waters Baseline 810 software package. Each kinetic run using ethylmethyl(*p*-nitrophenyl)sulfonium salt as a substrate was performed twice. Sample reaction mixtures for each kind of experiment (without and with a competitive inhibitor) follow. For host-catalyzed dealkylation of **12**, the reaction mixture consisted of 80 μL of sulfonium salt stock solution (3.80 mM), 30 μL of KHP stock solution, 120 μL of host stock solution, 30 μL of KSCN stock solution, and 240 μL of buffer. The uncatalyzed reaction used 120 μL of buffer instead of host stock solution. The reaction mixtures for the competitive inhibition study consisted of 70 μL of ethylmethyl(*p*-nitrophenyl)sulfonium stock solution, 30 μL of KSCN stock solution, 30 μL of KHP stock solution, 210 μL of 5-nitroquinoline stock solution, and 160 μL of host stock solution. For the uncatalyzed reaction, 160 μL of buffer was added instead of host solution.

For each experiment, the buffered pH = 9 solution of substrate, inhibitor (if any), internal standard, and host (for catalyzed reactions) was prepared without nucleophile in an Eppendorf tube and cooled to −5 °C in a salt–ice-water bath. The solution of nucleophile (chilled) was added, and the tube was shaken vigorously just prior to the first injection of sample. The tube was then placed in an oil bath maintained at 46 °C by a Thermo Watch. At each time point, the reaction mixture was cooled to −5 °C, and a 20-μL aliquot was removed and neutralized with 20 μL of pH = 7 phosphate buffer. The 40-μL sample was injected onto the column, and the reaction mixture was returned to the oil bath. A gradient elution was used to separate the reaction mixture components. Solvent A was H_2O, 0.1% TFA by volume; solvent B was acetonitrile, 0.1% TFA by volume. Elution was performed at 1.8 mL/min using a linear gradient from 20% to 100% solvent B from 0 to 10 min, maintained at 100% B from 10 to 12 min, brought back to 80% solvent A from 15 to 17 min, and washed from 17 to 35 min with 80% solvent A. Compounds were detected at 254 and 230 nm. Generally, KSCN eluted with the void volume, the KHP standard eluted at 3.3 min, the sulfonium salt eluted at 5.3 min, and the host eluted at 6.5 min. A calibration consisting of measured relative peak areas of five samples of various sulfonium salt concentrations and fixed KHP concentration was used to convert peak areas to concentrations.

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Mechanism of Decomposition of (*E*)-Methanediazoate in Aqueous Solutions†,[1]

Jari Hovinen, Jari I. Finneman, Surya N. Satapathy, Jian Ho, and James C. Fishbein*

Contribution from the Department of Chemistry, Wake Forest University, Winston-Salem, North Carolina 27109. *Received July 15, 1992*

Abstract: Rate constants for the decomposition of (*E*)-methanediazoate (**1**) at 25 °C, ionic strength 1 M ($NaClO_4$), are independent of the concentration of nucleophile in up to 0.25 M propylamine base, 0.5 M methoxyamine base, 0.20 M thiosulfate dianion, and 0.50 M azide ion. When the CH_3 group of **1** is transferred to benzoate ions, benzyl alcohol, and water upon decomposition in near neutral D_2O solutions, the isotopic integrity of the methyl group is maintained to the extent of 70–90%. The rate constant for the decomposition of protonated **1** in ethanol is 680 times slower than that in aqueous 4% ethanol under identical buffering and ionic strength conditions. Trapping of the methyl group derived from **1** by two pairs of nucleophiles gives the same ratios of methylated products as those determined from the decomposition of diazomethane. The solvent deuterium isotope effect for the decomposition of the protonated form of **1** is $k_{H_2O}/k_{D_2O} = 1.49 \pm 0.09$, and the activation parameters for its decomposition are $\Delta H^{\ddagger} = 69.6 \pm 1.5$ kJ/mol and $\Delta S^{\ddagger} = -4.5 \pm 9.5$ J/deg mol. It is concluded that **1** decomposes via equilibrium protonation on oxygen with subsequent rate-limiting cleavage of the O–N bond of the diazoic acid to yield the methanediazonium ion. This conclusion and measurements of the rate constants for decomposition of diazomethane under comparable conditions (25 °C, aqueous 5% acetonitrile, ionic strength 0.20 M) permit the first semiquantitative description of the decomposition of a simple alkanediazoate and associated intermediates in wholly or predominately aqueous media.

Introduction

Alkanediazoates are believed to be intermediates that are central to the DNA alkylating activity of a large class of *N*-alkyl-*N*-nitroso compounds that are carcinogens and/or cancer-chemotherapeutic agents.[2] Simple syn and anti alkanediazoates were synthesized separately nearly 100 years ago.[3] Alkanediazoates are generally unstable in aqueous solutions, decomposing with the evolution of nitrogen gas, though some of the anti forms are reportedly stable in cold water.[4] Some anti forms are known qualitatively to be more stable than the analogous syn forms.[4]

We recently reported the first rate constants for the decomposition of a simple alkanediazoate (**1**) in aqueous media at physiological pH.[5] On the basis of the pH–rate profile for the decomposition of **1** and the observed 92% yield of methanol, it was deduced that the mechanism of decomposition of **1** involves a rate-limiting reaction of the protonated diazoate D–H, as in eq 1. A minimal mechanism was presented in light of the well-known complexity of the mechanisms of decomposition of analogous arenediazoates.

There is a diversity of opinion about the mechanisms by which simple alkanediazoates decompose. Recent theoretical studies[6]

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(2) Lawley, P. D. In *Chemical Carcinogens*: Searle, C. D., Ed.; ACS Monograph Series 182; American Chemical Society: Washington, DC, 1984.

(3) (a) Thiele, J. *Justus Liebigs Ann. Chem.* **1910**, *376*, 239. (b) Hantzsch, A.; Lehmann, M. *Chem. Ber.* **1902**, *35*, 897.

(4) Moss, R. A. *Acc. Chem. Res.* **1974**, *7*, 421 and references within.

(5) Hovinen, J.; Fishbein, J. C. *J. Am. Chem. Soc.* **1992**, *114*, 366.

0002-7863/92/1514-10321$03.00/0